Immunology and infectious diseases

Label-free interaction analysis: revealing the secrets of biomolecular interactions
Biacore systems from GE Healthcare

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Introduction

Despite the unprecedented quality of health among present day populations of industrialized countries, the prevention and management of certain chronic illnesses and cancers continue to present challenges. The search for effective treatments for diseases such as multiple sclerosis, for which a causative agent has yet to be unambiguously identified, is proving particularly difficult while the ability of the human immunodeficiency virus to continually adapt and evade novel therapies may continue to be a major health threat in the 21st century.

Technologies for the analysis of molecular interactions are needed across the entire spectrum of clinical science, from the need to understand the mechanisms underlying pathological events, to the validation of drugs and antibodies in clinical trials. This booklet contains short summaries of selected published work that demonstrate the broad utility of label-free interaction analysis in laboratories from commercial research facilities searching for novel therapeutics to small hospital units seeking improved diagnostic procedures.

Inflammation

Measuring acute phase protein glycosylation patterns as a biomarker for inflammation

- Assay is sufficiently robust to handle crude clinical samples
- Response is specific to glycosylation levels and is independent of the total amount of captured glycoprotein
- A more rapid alternative to chemical, enzymatic and chromatographic techniques

The glycosylation pattern of proteins is known to change over the course of inflammatory responses to pathological events and injury. Mathias Liljeblad and co-workers at Linköping University in Sweden have shown how a Biacore assay can be used to measure glycosylation patterns of the acute phase protein, α1-acid glycoprotein, a marker of inflammation in severe burns patients (1).

EDTA-treated serum from patients was diluted and injected over polyclonal antibodies to α1-acid glycoprotein (AGP) immobilized on a sensor surface and bound analyte was detected using a lectin. Before immobilization, the antibodies were incubated with sodium periodate to oxidize terminal fucose residues and so reduce unwanted binding.

The assay developed by Liljeblad et al. had to be sufficiently robust to handle crude clinical samples and it was therefore vital to ensure that other serum proteins did not bind to the sensor surface and possibly interfere with the lectin: AGP interaction. The importance of validating experimental protocols under “real” conditions should not be underestimated, especially where the injected sample is complex e.g. clinical samples or crude bacterial lysates.
AGP samples with a known fucosylation of between one and five fucose units per molecule were analyzed. The saturation response was linear across the entire range of fucosylated analytes; as the total amount of AGP was constant in each case, the response was shown to be dependent purely on fucosylation levels (Fig 1).

In order to quantify the fucosylation level of AGP in serum samples from patients, antibodies were immobilized on a sensor surface at low density and a fucosylation ratio was established by dividing the saturation response after addition of lectin by the response due to captured AGP alone, before the addition of serum. In this way it was possible to determine the extent of fucosylation independently of the total amount of captured AGP. The assay was used to assess AGP fucosylation over time in patients with acute inflammation caused by severe burns. Fucosylation ratios derived from Biacore assays correlated closely to those obtained from an ELISA. One advantage of using Biacore assays, however, is that by measuring a fucosylation ratio, knowing the total amount of captured AGP (which the ELISA assay does not detect) is not critical. This may be important when measuring post-translational modifications in proteins that are only available in quantities below which it is possible to saturate a solid phase surface (Fig 2). Biacore systems were shown to be a viable and rapid alternative to chemical, enzymatic and chromatographic techniques for analyzing transient carbohydrate modifications to marker proteins in a clinical setting.

Reference
Investigating how poxviruses and herpes viruses inhibit the human chemokine network

- Develop antagonists for immune cell chemotaxis to counter inflammation
  - Find out if viral chemokine inhibitors and natural chemokine GPCRs recognize overlapping epitopes
  - Define the stoichiometry of interactions between chemokines and viral chemokine inhibitors

Chemokines are a class of small, secreted proteins that stimulate leukocyte chemotaxis to areas of inflammation. Poxviruses and herpes viruses inhibit the chemokine network by producing chemokine-binding proteins that competitively inhibit interactions between chemokines and their natural receptors. In the work cited below, these inhibition mechanisms were investigated using label-free protein interaction analysis.

Poxviruses and herpes viruses inhibit the chemokine network by producing chemokine-binding proteins (CKBPs) that competitively inhibit the typically high affinity interactions between chemokines and their cognate GPCRs or low affinity interactions with glycosaminoglycans (GAGs). Poxvirus CKBP-II prevents the CC class of chemokines from binding to cell surface receptors. In two independent studies, the group of Grant McFadden at the John P. Robarts Research Institute in London, Ontario (2) and that of Roman Urfer at Novartis Pharma AG in Basel (3) used a Biacore system to test the interactions between CKBP-II and a panel of point-mutated versions of the human CC-chemokine, MCP-1.

![Fig 3. Interaction profiles of wildtype and mutant MCP-1 to immobilized CKBP-II protein (Figure reproduced with permission from Seet and McFadden, J. Leukoc. Biol. 72, 24-34 [2002]). CKBP-II: Fc fusion protein is immobilized on a sensor surface and a titration series of chemokines is serially injected over the prepared surface. The plots demonstrate that the binding responses have similar saturation levels, suggesting that CKBP-II binds a monomeric form of MCP-1.](image-url)
Both studies showed that the affinity of viral CKBP-II for wildtype chemokine MCP-1 was similar to that of MCP-1 for its natural cognate GPCR, suggesting that even at low concentrations, CKBP-II may behave as an antagonist. A Biacore system was also used to work out the stoichiometry of the interaction between MCP-1 and CKBP-II by immobilizing CKBP-II on a sensor surface and injecting a titration series of wildtype dimeric MCP-1 or a monomeric MCP-1 mutant over the prepared surface. The interaction profiles derived from both experiments had similar saturation levels, suggesting that CKBP-II could bind the monomeric form of MCP-1 (Fig 3). These studies also demonstrated that CKBP-II binds epitopes on MCP-1 that are recognized by CCR2B, illustrating the concept of convergent evolution between viruses and their hosts at the molecular level.

It is believed that chemokines may exacerbate some diseases by causing excessive inflammation, directing immune cells to sites of trauma. Viral CKBP with broad chemokine specificity may therefore be attractive candidates for antagonists of immune cell chemotaxis. Trials in animal models have shown that in certain cases, administration of CKBP does in fact hinder inflammatory cells from reaching specific organs while leaving systemic immune responses unaffected.

References
2. Seet, B. T. et al.
   Molecular determinants for CC-chemokine recognition by a poxvirus CC-chemokine inhibitor.
   The viral CC-chemokine binding protein vCCI inhibits monocyte chemoattractant protein-1 activity
   by masking its CCR2B binding site.

Validating “gene leads”
- Determine the affinity of selected protein interactions from large scale screens

AGT Biosciences is an Australian biotechnology company that has enhanced its established suite of leading edge technologies with a Biacore system from GE Healthcare to validate their many gene leads in the fields of diabetes, obesity and depression (4).

The AGT Biosciences group of Professor Greg Collier used differential PCR to reveal expression of a novel gene in the liver of fasting rats that spontaneously develop a type 2 diabetes-like disease. Sequence analysis of the gene suggested a membrane-embedded protein, Tanis. A positive correlation between tanis expression and levels of circulating triglycerides, blood glucose and insulin suggested that tanis may be important in the pathology of type 2 diabetes, a hypothesis further supported by the fact that tanis expression in cultured hepatocytes decreased with the addition of glucose.

Walder et al. used a yeast two hybrid screen to search for proteins that interacted with tanis from an expression library derived from human liver cDNA. Three clones encoding alternate transcripts of serum amyloid A (SAA), an acute phase inflammatory response protein expressed mainly in liver, were identified as tanis ligands. As high circulating SAA is prognostic of type 2 diabetes and is released into the circulation in complex with HDL cholesterol, the authors suggested a functional link between type 2 diabetes and inflammation.
To confirm the yeast two hybrid data and to find out if native SAA purified from human plasma was able to bind tanis, binding profiles of the interactions with native and recombinant SAA were characterized using a Biacore system. Both plasma-purified SAA and recombinant SAA bound recombinant tanis. Binding was even seen when using recombinant tanis that lacked a membrane-spanning domain and the entire sequence of adjacent N-terminal amino acids, suggesting that the binding site for SAA lies within the C-terminal region.

These data support the concept that tanis may be important in the development of diabetes and that it may be a promising therapeutic target. Studies using human liver cells have shown that increased expression of tanis causes the cells to become refractive to insulin, a possible reflection of the resistance to insulin seen in diabetes.

Reference


Parasitology

Understanding how insect vectors inhibit the host blood coagulation system to optimize transmission of parasites

- Biacore analysis shows that insect salivary protein inhibits the extrinsic coagulation pathway by binding to the Gla domain of Factor IX
- A lipid monolayer on a sensor surface is used to show that insect salivary protein inhibits the intrinsic coagulation pathway by preventing the interaction of Factor IX with Factor XIa or by inhibiting insertion of Factor IXa into the plasma membrane

*Rhodnius prolixus*, a blood-sucking insect also known as the kissing bug, is a vector in the lifecycle of the protozoan parasite, *Trypanosoma cruzi*, the causative organism of American trypanosomiasis, a life-threatening disease endemic in South and Central America. Once transmitted to the host, *T. cruzi* multiplies inside the muscle cells of the heart causing severe weakening. The group of Masao Yuda at Mie University in Tsu used label-free protein interaction analysis to understand how *Rhodnius prolixus* manages to efficiently transmit parasites by inhibiting host blood coagulation, prolonging the time that the insect can feed (5). A 20 kDa heme-binding protein called prolixin-S has been isolated from the insect’s salivary glands and has been shown to be an anti-coagulant, a function in which the heme group appears to play no part. How then, does prolixin-S exert its anti-coagulation effects? Isawa et al. tested the binding of prolixin-S to various components of the coagulation cascade. Factor IX was of particular interest because the activation of this protein from its zymogenic form to its active form (Factor IXa) is common to both the intrinsic (foreign surface-induced) and extrinsic coagulation cascades (Fig 4).
Factor IX or Factor IXa were immobilized on a sensor surface and a titration series of recombinant prolixin-S was injected over the prepared surface. Kinetic and affinity data from these experiments are summarized in Table 1. Prolixin-S was shown to bind both the zymogenic and active forms of Factor IX with a similar affinity and kinetic profile. An important result derived from these Biacore experiments was that prolixin-S did not bind to Factor IXa previously digested with chymotrypsin and thus void of the Gla domain, indicating that this domain may be the binding site for prolixin-S.

### Table 1. Rate and affinity constant measurements of interactions between prolixin-S and Factor IX/IXa

<table>
<thead>
<tr>
<th>Immobilized binding partner</th>
<th>Association rate constant ($k_a$)</th>
<th>Dissociation rate constant ($k_d$)</th>
<th>Affinity dissociation constant ($K_D$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor IX</td>
<td>$5.0 \times 10^5$ M$^{-1}$ s$^{-1}$</td>
<td>$6.5 \times 10^{-3}$ s$^{-1}$</td>
<td>13.0 nM</td>
</tr>
<tr>
<td>Factor IXa</td>
<td>$3.1 \times 10^5$ M$^{-1}$s$^{-1}$</td>
<td>$4.1 \times 10^{-3}$ s$^{-1}$</td>
<td>13.2 nM</td>
</tr>
</tbody>
</table>

Propagation of the extrinsic coagulation pathway requires that Factor IXa, Factor VIIIa and Ca$^{2+}$ form a complex at a membrane interface to form Xase, a multicomponent enzyme that activates Factor X and leads ultimately to clot formation. The effect of prolixin-S on the interaction between Factor IXa and lipid membranes was assessed by injecting Factor IXa over a lipid monolayer on a sensor surface in the presence of prolixin-S. The data showed that binding of Factor IXa to the lipid monolayer was progressively inhibited by increasing concentrations of prolixin-S.

### Reference
Learning how the regulated expression of oligosaccharides helps parasites evade the immune systems of host organisms

- Rank antibodies to parasite antigens according to affinity to select prospective diagnostic reagents
- Detect immunoglobulin isotype switching during parasite infection in humans
  - Match isotype patterns to patient disposition to diagnose stage of infection

Schistosomiasis is endemic throughout large parts of South America, sub-Saharan Africa and the Far East. The disease is caused by parasitic worms and affects some 200 million people worldwide. The parasite requires both humans and a particular species of fresh water snail as host at different stages of its life cycle. Alexandra van Remoortere and colleagues at the Leiden University Medical Center and the Vrije Universiteit have studied the expression of fucosylated oligosaccharides during different maturation stages of *S. mansoni* to learn how the parasite evades the immune systems of both organisms.

The initial humoral immune response mounted against schistosome infection is often directed against a gut-associated antigen expressing repeating units of the fucose-containing trisaccharide, Lewis^x^ (Le^x^). van Remoortere *et al.* studied the expression and specificity of fucosylated oligosaccharides during different maturation stages of *S. mansoni* using a panel of monoclonal antibodies in a primary screen using an ELISA. Binding antibodies were then affinity ranked using label-free protein interaction analysis to select the most promising diagnostic reagents (6).

The group of André Deelder at the Leiden University Medical Center has studied the isotype dynamics of the immune response in the sera of infected patients in Kenya and the Philippines, two countries in which the disease is endemic (7). Sera from patients were injected over synthetic glycoprotein conjugates immobilized on a sensor surface. By sequentially running two isotype-specific antibodies, the isotype of the antibodies in the immune response were then determined (Fig 5).

![Figure 5](image_url)

**Fig 5.** Interaction profiles illustrating the interaction of serum antibodies with Galβ1-4GlcNAc (LDN), Galβ1-4(Fucα1-3)GlcNAc (Le^x^) or fucosylated LDN in patients infected with *S. japonicum* (A) or *S. mansoni* (B). The times of start of injection of serum (S), followed by anti-human IgM (M), anti-human IgG (G) and regeneration with 100 mM HCl (R) are indicated by arrows (Figure reproduced with permission from van Remoortere *et al.*, *Infect. Immun.* **69**, 2396–2401 (2001)). The clearly distinct interaction profiles, where IgG responses predominate during *S. japonicum* infection while IgM predominates during *S. mansoni* infection show that a Biacore assay may be used to differentiate between infections caused by similar parasites.
Antibodies to one particular epitope, the parasite-specific fucosylated structure, GalNAcβ1-4(Fucα1-2Fucα1-3)GlcNAc (LDN-DF) were abundant in the sera of all patients regardless of the infecting schistosome species. When the data was grouped in order to visualize the antibody response to LDN-DF according to isotype, patient age and response to each of the three main schistosome species, response patterns were discerned, suggesting that this Biacore-based assay may be useful in diagnosis of schistosomiasis (Fig 6).

References

6. van Remoortere, A. et al.
Various stages of schistosoma express Lewis'α, LacdiNac, GalNAcβ1-4(Fucα1-3)GlcNac and GalNAcβ1-4(Fucα1-2Fucα1-3)GlcNac carbohydrate epitopes: detection with monoclonal antibodies that are characterized by enzymatically synthesized neoglycoproteins.

7. van Remoortere, A. et al.
Profiles of immunoglobulin M (IgM) and IgG antibodies against defined carbohydrate epitopes in sera of schistosoma-infected individuals determined by surface plasmon resonance.
**Rheumatology**

*Showing how analysis of interaction kinetics can help explain why symptoms of rheumatic diseases are often more severe at low temperatures*

- Understand how temperature affects protein interactions and may impact on the symptoms of rheumatoid arthritis

Rheumatoid factor is an autoimmune antibody that targets host IgG in patients with rheumatoid arthritis, the symptoms of which are frequently exacerbated in cold climates. The formed immune complexes are prevalent in synovial fluid and are associated with chronic tissue damage in the rheumatoid synovium. The group of Naoya Kojima at Tokai University in Kanagawa used label-free protein interaction analysis in a kinetic analysis of interactions involving rheumatoid factor over a range of temperatures (8). Their results suggest why the symptoms of rheumatoid arthritis may be particularly severe at low temperatures.

Matsumoto et al. prepared rheumatoid factor (RF) of different isotypes from the pooled sera of rheumatoid arthritis patients. A Biacore assay was set up in which IgG was immobilized on a sensor surface. RF was then injected over the prepared surface at various temperatures and the isotypes were detected after a subsequent injection of anti-isotype antibodies. Kinetic analysis of the interactions showed that IgG-RFs had very different binding profiles compared with RFs of alternative isotypes. For example, interactions with IgG were profoundly affected as the temperature increased from 6°C to 30°C, with a reduction in association rate constant from $10.7 \times 10^4$ M$^{-1}$s$^{-1}$ at 6°C to total abrogation of binding at 30°C. The association rates of other isotypes were only marginally affected by changes in temperature over this range. The dissociation rate of IgG-RF was more rapid than that of either IgM-RF or IgA-RF but fell sharply at temperatures above 25°C. The authors suggest that once formed at low temperature, immune complexes containing IgG-RF are stable and dissociate slowly, remaining in the synovial fluid for a considerable time and possibly causing synovial lesions (Fig 7 and Table 2).

**Reference**

Understanding the origins of autoantibody specificities in autoimmune diseases

- Detect the presence of novel epitopes formed by the association of discrete antigens, leading to autoimmune reactions

Ro is a ribonucleoprotein associated with RNA and is one of several targets of autoantibodies in the serum of patients with systemic lupus erythematosus (SLE). Antibodies to Ro protein were affinity purified from serum and were found to bind both 52 kDa and 60 kDa transcripts of Ro protein. Binding to the 60 kDa form of Ro could be inhibited by either the 52 kDa or the 60 kDa form, suggesting that the antibody bound an epitope common to both forms.

The group of Hal Scofield at the Oklahoma Medical Research Foundation and the University of Oklahoma Health Sciences Center screened antibodies to 52 kDa Ro against a set of overlapping peptides on 60 kDa Ro by ELISA (9, 10). Four identified epitopes were then synthesized and immobilized on a sensor surface. The highest affinity interaction involved an epitope that was recognized by antibodies purified from all patient sera tested.

Sera from SLE patients bound as many as 11 epitopes on 60 kDa Ro but purified anti-52 kDa Ro bound only to one epitope. To test their hypothesis that one epitope on 60 kDa Ro associated with another on 52 kDa Ro to form a novel binding site, the authors screened interactions between amino acids 197 and 207 of 52 kDa Ro with each of the identified peptides from 60 kDa Ro. This peptide sequence bound three of the four peptides identified by the anti-52 kDa Ro antibody on 60 kDa Ro, suggesting that the 197-207 region on 52 kDa Ro peptide interacted with several peptides on 60 kDa Ro. The authors suggest the two Ro forms associate to form a conformational epitope. It may also explain why, in SLE, antibodies to 52 kDa Ro are nearly always found together with anti-60 kDa Ro.

References
Using label-free interaction analysis to form a model for the generation of autoantibodies in autoimmune diseases

- Identify and define antigen/antibody/anti-idiotype networks in autoimmune diseases
- Use data from antibody specificity studies to help understand how autoantibodies contribute to disease pathology

Although anti-idiotype antibodies to anti-DNA have been found in SLE, their significance is unclear. An SLE-like condition can be induced in mice immunized with “idiopeptides” that mimic the framework region and CDR regions of anti-DNA antibodies. A collaborative group at King’s College London - Elvira Eivazova, Brian Sutton, Jim McDonnell and Norman Staines - hypothesized that anti-DNA antibodies may be induced both by DNA and by anti-idiotype antibodies (11, 12) that induce further production of anti-DNA antibodies in a feedback loop (Fig 8).

An intriguing feature of one mouse model with spontaneously occurring SLE is the apparently coordinated appearance of two autoantibody populations; one to double stranded DNA and another to idiotopes on exposed faces in the CDR and framework regions of these antibodies. In order to investigate the interplay between DNA, anti-DNA and anti-idiotype networks in SLE models, Eivazova et al. synthesized “idiopeptides” from a monoclonal antibody to double stranded (ds) DNA. Mice immunized with these peptides generated not only anti-idiopeptide antibodies (see Table 3) but also antibodies to dsDNA.

It should be noted that although antibodies raised against different idiopeptides bound the parent antibody, they were also highly specific for the primary immunogens. Cross reactivity of the anti-idiopeptide antibodies for dsDNA was confirmed in a label-free protein interaction assay in which affinity-purified anti-idiopeptide antibodies were pre-incubated with dsDNA before injection over idiopeptides immobilized on a sensor surface. Results showed dose-dependent inhibition by DNA of antibody binding to its “natural” target (Fig 9).

Two mouse models for human SLE develop anti-DNA antibodies as they age. The specificities of the autoantigens were identified by immobilizing synthetic double or single stranded oligonucleotides on a sensor surface. Monoclonal antibodies (one from a spontaneous SLE strain generating a monoclonal antibody against single stranded (ss) and ds DNA and another generating only anti-ssDNA) were purified from ascitic fluid and purified on protein G. Preference for particular nucleotides were seen in both dsDNA or ssDNA contexts. One antibody was identified that exclusively recognized ssDNA while another showed faster association to and slower dissociation from polyGC than polyG or polyC (Table 4). These results may clarify a suspected pathologic role of autoantibodies in SLE; that while the profile of anti-dsDNA antibodies closely follows disease progression, the emergence of anti-ssDNA antibodies follows apoptosis and degradation (strand separation) of DNA following release from the cell.

References
Fig 8. A possible feedback mechanism regulating sustained stimulation of B-cell clones producing autoanti-DNA antibodies.

Fig 9. Inhibition of binding of anti-p64 antibodies to immobilized peptide by dsDNA. Affinity-purified anti-p64 antibodies were incubated for five hours with dsDNA at the concentrations indicated. The mixtures were then injected over immobilized p64. Results are given as percentage reduction in antibody binding obtained with antibody in the absence of DNA. dsDNA by itself did not bind to immobilized p64. Figure reproduced with permission from Eivazova et al., Arthritis Rheumatism 43, 429-439 (2000). Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

Table 3. Summary of kinetic and affinity data for binding between affinity purified (a.p.) antibodies to anti-DNA antibody-derived idiotopes (idiotopeptides) or to parent anti-DNA antibody

<table>
<thead>
<tr>
<th>Immobilized binding partner</th>
<th>Binding partner in solution</th>
<th>Association rate constant ($k_a$)</th>
<th>Dissociation rate constant ($k_d$)</th>
<th>Affinity dissociation constant ($K_D$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiotope p64</td>
<td>a.p. anti-p64</td>
<td>$1.1 \times 10^4$ M$^{-1}$s$^{-1}$</td>
<td>$1.5 \times 10^4$s$^{-1}$</td>
<td>13.6 nM</td>
</tr>
<tr>
<td>Anti-DNA</td>
<td>a.p. anti-p64</td>
<td>$5.9 \times 10^3$ M$^{-1}$s$^{-1}$</td>
<td>$8.4 \times 10^3$s$^{-1}$</td>
<td>142 nM</td>
</tr>
<tr>
<td>Anti-DNA</td>
<td>Serum anti-p92</td>
<td>$3.1 \times 10^3$ M$^{-1}$s$^{-1}$</td>
<td>$1.3 \times 10^3$s$^{-1}$</td>
<td>420 nM</td>
</tr>
</tbody>
</table>

Table 4. Summary of kinetic and affinity data for binding between affinity purified (a.p.) antibody V-88, a spontaneously occurring antibody to DNA in SLE-susceptible mice.

<table>
<thead>
<tr>
<th>Immobilized binding partner (DNA)</th>
<th>Association rate constant ($k_a$)</th>
<th>Dissociation rate constant ($k_d$)</th>
<th>Affinity dissociation constant ($K_D$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC25</td>
<td>$2.9 \times 10^3$ M$^{-1}$s$^{-1}$</td>
<td>$5.3 \times 10^4$s$^{-1}$</td>
<td>18 nM</td>
</tr>
<tr>
<td>C25</td>
<td>$1.1 \times 10^4$ M$^{-1}$s$^{-1}$</td>
<td>$1.3 \times 10^3$s$^{-1}$</td>
<td>118 nM</td>
</tr>
<tr>
<td>G25</td>
<td>$1.1 \times 10^4$ M$^{-1}$s$^{-1}$</td>
<td>$1.2 \times 10^3$s$^{-1}$</td>
<td>109 nM</td>
</tr>
</tbody>
</table>
Label-free interaction analysis in basic clinical research: revealing the hidden details

*Identifying the receptors responsible for the uptake of environmental toxins*

- Use label-free interaction analysis to differentiate the roles of receptors that appear to have similar functions in cell-based assays

Cubilin and megalin have broad ligand specificity and have been proposed as scavenger receptors. They have recently been identified as receptors for Clara Cell Secretory Protein (CCSP). The physiological role of CCSP is of great interest because of its capacity to bind polychlorinated biphenyl metabolites (PCBs), industrial chemicals which although forbidden for many years, are still detected in human tissues. It is therefore possible that cubilin and megalin are responsible for the uptake of PCBs bound to CCSP.

The Group of Thomas Willnow and colleagues at the Max-Delbrueck-Center for Molecular Medicine in Berlin showed using label-free interaction analysis that cubilin bound CCSP with very high affinity and that this interaction was blocked if cubilin was pre-incubated with the receptor antagonist, RAP [13]. CCSP, however, did not bind megalin, despite data from functional studies suggesting that antibodies against megalin blocked internalization of CCSP. The data from the functional studies together with those from Biacore assays suggest a dual receptor system where CCSP is bound by cubilin but where uptake is mediated by megalin. The authors speculate that the tissue uptake and toxicity of PCBs may be partly explained by the fact that PCBs displace physiological ligands from CCSP and enter cells that express endogenous CCSP receptors cubilin and megalin.

Reference

Treatment regimes and clinical trials

**Optimizing the performance of conjugated drugs**

- Select fusion partners to create conjugates with desirable drug: carrier interaction profiles for optimal clinical effect

The efficacy of drugs is partly determined by the rate of elimination from the body. One way to counter rapid clearance by the kidneys is to conjugate the drug with a large molecule such as albumin. Although conjugation increases the time a substance remains in circulation, an important consideration is the fact that drugs associated with plasma proteins are often impeded in binding their intended target. A balance, therefore, must be struck between delayed clearance and a reasonable rate of dissociation of the drug from the carrier during the time that has been “won”.

Mark Dennis and colleagues at Genentech Inc., South San Francisco used label-free protein interaction analysis to rank peptides according to their affinity for albumin (14). High affinity binders were fused with Fab protein to create a complex that could bind albumin without interfering with the antigen binding properties of Fab. The work of Dennis *et al.* shows how Biacore systems may be used to select fusion partners in applications such as the development of tumor targeting and imaging reagents where a sufficient time of exposure is required for adequate adsorption into the tumor.

**Reference**


**Testing the immunogenicity of therapeutic antibodies in clinical trials**

- Experience from using Biacore systems in clinical trials
  - Antibody response profiles predict the frequency of adverse events
  - Assay is sufficiently robust to handle untreated human serum
  - Unlike ELISA, the assay is not hampered by the fact that both the therapeutic reagent and the patient response to it are immunoglobulins from the same species

As therapeutic reagents, murine antibodies are limited due to immunogenic responses by the host. Investigators at the Ludwig Institute for Cancer Research in New York constructed a humanized version of a therapeutic murine monoclonal antibody recognizing a marker uniquely expressed in human colonic epithelium and colon cancer by grafting CDR regions into a human IgG framework. The hybrid antibody was then tested in phase I and phase II clinical trials in patients with colon cancer and immunogenicity was monitored using label-free protein interaction analysis (15).

Humanized antibody (huAb A33) was immobilized on a sensor surface and patient sera were injected over the prepared surface. Two broad groups of responders were identified. Type I responders were characterized as those in whose sera anti-huAb A33 appeared within 2 weeks and in which the anti-huAb A33 titer then declined despite continued huAb A33 treatment. A second, Type II group of patients responded more slowly and progressively over the course of continued therapy. In the latter group, physiological adverse events were more prevalent than in the Type I group and therapy with huAb A33 was discontinued in these patients.
Further, the isotype of antibody response by patients could be characterized using a Biacore sandwich assay. Briefly, pre-treatment of diluted serum with protein G (to remove IgG) abrogated the anti-huAb A33 response in all but a handful of patients while treatment with caprylic acid (to precipitate all non-IgG protein) had little effect. In the few patients in whom residual activity remained after protein G treatment of serum, a signal was detected after a subsequent injection of anti-IgM over the sensor surface.

Patients with Type II responses did not develop adverse physiological symptoms until several weeks after recording progressively increasing anti-huAb A33 responses in a Biacore assay. The investigators were therefore able to use label-free protein interaction analysis to predict the appearance of adverse events (Fig 10).

Reference

Further references describing how Biacore systems have been used in clinical trials