Immune interventions

Present when the T-cell receptor was first genetically characterised, Professor Arthur Vandenbark has since devoted his career to developing therapies based on their structures. Here, he discusses how his studies have led to a novel approach to target autoimmune and degenerative diseases.

What would you pick out as the highlights of your research career?

Since being witness to the discovery of the genetic design of the T-cell receptor (TCR) in the early 1980s, I have been fascinated by TCR structure, activation and function. Access to TCR protein sequences enabled investigation of their immunogenicity and regulatory functions and prompted the creation of the first single-chain major histocompatibility complex (MHC)/peptide construct, designed to ligate specific TCRs expressed by pathogenic T-cells (recombinant TCR ligand (RTL) technology).

The potency of RTL therapy for a variety of inflammatory conditions led to the fortuitous discovery that the DRI domain intrinsic to RTLs could bind to the CD74 molecule (the major macrophage migration inhibitory factor (MIF) receptor) and block the inflammatory effects of MIF that are involved in many autoimmune diseases, including multiple sclerosis (MS). Things have thus come full circle from my days as a PhD student measuring MIF activity using the migration of peritoneal macrophages.

To what extent do antigen-specific CD4+ T-cells mediate autoimmune diseases such as MS and rheumatoid arthritis?

Although there are many theories, it seems that autoreactive myelin or connective tissue specific T-cells, naturally present or driven by cross-reactivity with infectious organisms, arise in genetically predisposed individuals when natural immunoregulation fails. MHC class II molecules represent the biggest risk factor for MS. My aim is to link the risk alleles to autoreactive processes.

Why did RTLs become a particular focus for your research?

MHC class II/peptide complexes were thought to exist mainly on the antigen-presenting cell (APC), which also expresses co-stimulatory molecules that lead to full T-cell activation. Our initial goal was to produce a soluble, single-chain molecular construct that would retain the complementary shape for TCRs present on disease-causing T-cells, in order to track and modify their function. Thus, we designed an RTL molecule that contained only the
outermost domains of class II α and β chains from disease associated alleles, coupled with known pathogenic peptides. These constructs naturally folded into a very stable conformation that mimicked secondary structures of peptide-loaded four-domain class II MHC molecules.

**How important are MHC class II molecules to the immune response?**

Usually, protective CD4⁺ T-cell responses involve TCR interaction with MHC class II/peptide complexes on the APC. Different MHC class II molecules have different shaped binding clefts that favour binding of particular peptide motifs. One idea regarding disease risk factors, such as HLA-DR2 for MS, is that there may be certain disease-associated peptides that are optimally bound and presented to CD4⁺ T-cells by the DR2 class II allele.

**What success has the RTL approach seen in its ability to reverse or prevent autoimmune diseases?**

RTLs have demonstrated a remarkable ability to both prevent and reverse signs of autoimmune and inflammatory conditions in mice and rats. In our phase 1 clinical trial, we determined that the minimum detectable therapeutic dose of RTL1000 on experimental autoimmune encephalomyelitis (EAE), with a single injection, was less than 1 µg. That number was even smaller with repeated doses. This is pretty incredible for a biologic molecule. Other RTLs designed for different applications were all very active. We are now turning our attention to RTL effects in chronic EAE, and have found that RTL therapy can reverse myelin and axonal damage in extremely ill animals. This is very encouraging and provides a strong rationale for the treatment of progressive MS and other neurodegenerative conditions.

**Finally, how might industrial collaboration help you to achieve your ambitions?**

Currently, Artielle Immunotherapeutics, Inc., which is funded through Sanderling Ventures and the holder of exclusive worldwide licenses for the RTL technology, has enabled the successful completion of the phase 1 study and the initiation of a phase 2 study to obtain proof-of-concept data in secondary progressive MS (SPMS). Our effort to bring RTL1000 into the market place as a first line drug for SPMS, as well as application of the RTL technology to other diseases, could be expedited through investments and partnerships with other pharmaceutical companies.

**Designer molecules**

Researchers at Oregon Health & Science University and Portland Veterans Hospital have developed recombinant T-cell receptor ligands, unique proteins that have been shown to reverse inflammation in the brain and promote neuronal repair. Already in clinical trials, it holds great potential as a promising new treatment for multiple sclerosis.

**Autoimmune diseases, which occur when the body’s immune system attacks its own tissue, are some of the most complex and devastating diseases known to medicine.** Thought to require genetic predisposition and an environmental trigger, these diseases can destroy diverse tissues, leading to a host of conditions including multiple sclerosis (MS), rheumatoid arthritis and type 1 diabetes.

**Help**er T-cells, also called CD4⁺ T-cells, could be a central component in the pathogenesis of autoimmune diseases. CD4⁺ T-cells are activated when they are presented with small fragments of antigens by major histocompatibility complex (MHC) class II molecules, that are expressed on the surface of antigen-presenting cells (APCs). The APC interaction with the T-cell receptor (TCR) induces rapid division and cytokine secretion, which enhances the active immune response.

In the context of autoimmune disease, CD4⁺ T-cells are thought to direct the disease towards its target tissue, where they recognise a normal protein (autoantigen), such as myelin, as foreign and produce pro-inflammatory cytokines. This leads to the recruitment and activation of other immune cells called lymphocytes and monocytes, that destroy the target tissue.

**Professor Arthur Vandenbark, a member of Oregon Health & Science University (OHSU)’s Multiple Sclerosis Center, was first inspired by early experiments which showed that stimulating the TCR via MHC class II molecules caused CD4⁺ T-cells to become unresponsive to the antigen. This demonstrated that a complete ligand (antigen plus MHC), in the absence of co-stimulatory signals normally provided by APCs, could achieve immunosuppression – a potential new treatment for autoimmune disease. Building on these findings, Vandenbark’s lab has since developed a new class of extremely potent MHC constructs.** Termed recombinant TCR ligands or RTLs, they can prevent and even reverse autoimmune diseases in mice, mainly by blocking the binding of a key cytokine – macrophage migration inhibitory factor (MIF) – to its CD4⁺ receptor on monocytes.

**Models of MS**

To conduct his studies, Vandenbark primarily uses experimental autoimmune encephalomyelitis (EAE), an animal model of the human demyelinating disease MS. The model shares the neuropathology and immunological dysfunction of MS and can be used to study the autoimmune processes directed at the central nervous system (CNS) even to the level of the cell types and inflammatory factors involved.

EAE is induced by injecting mice with myelin proteins, which promotes a robust CD4⁺ T-cell (Th1) response. As a result, ascending paralysis, axonal damage and demyelination develop. “Over the years, many nuances of the EAE model have been uncovered that allow us to study the associated processes such as demyelination and nerve damage,” Vandenbark elaborates. Based on a sound understanding of the structure of the MHC class II molecule, Vandenbark’s laboratory has designed RTLs that can prevent and even reverse damage in these models.

**Molecular mimicry**

MHC describes a group of molecules that control much of the function of the immune system. MHC molecules bind antigens and display them on the cell surface in order for T-cells to recognise them and trigger an immune response. MHC class II molecules specifically present antigens derived from extracellular proteins. These membrane bound glycoproteins are made up of loosely linked α- and β-polypeptide subunits, each of which comprises a cytoplasmic tail, a single membrane-spanning sequence, and two extracellular domains. Peptides from the processed antigen bind in the pocket formed by the α1 and β1 domains of the molecule.

In order to develop a simple and effective soluble reagent that could selectively bind the TCR, Vandenbark’s team designed molecules
INTELLIGENCE

RECOMBINANT T-CELL RECEPTOR LIGAND (RTL)

OBJECTIVE
To develop a novel, efficacious biologic for treatment of autoimmune diseases, particularly multiple sclerosis.

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ARTHUR A VANDENBARK received an AB degree from Stanford University and MS and PhD degrees from Washington State University. After a postdoctoral fellowship at Oregon Health & Science University (OHSU), he has since worked as a Career Scientist for the US Department of Veterans Affairs and as a professor at OHSU studying immunoregulatory mechanisms in autoimmune diseases.

consisting of these two domains linked to the myelin peptide in a single polypeptide chain; the proteins can be easily produced in _Escherichia coli_ and are highly stable. After purification and folding, the RTL takes a shape similar to the distal domains of the full-length class II MHC molecule containing its bound peptide. Their stability and ability to inhibit both T cells and monocytes make RTLs a very useful template for treating CD4+ T cell-mediated autoimmune diseases.

This approach has shown itself to be extremely versatile. “Using this platform, we have constructed dozens of different MHC class II/peptide combinations for preventing and treating different diseases in rodents, including models of EAE, arthritis and even stroke,” Vandenbark elucidates.

Based on this promising preclinical data, Artielle Immunotherapeutics, Inc., backed by Sanderling, took on the technology in 2004. Since then, their main focus has been the MHC class II molecule RTL1000, which contains the human MOG-35-55 peptide (that induces EAE in rodents) linked to the MS risk factor HLA-DR2b.

**UNEXPECTED REVELATIONS**
The team recently completed a phase 1 clinical trial of RTL1000. The study enrolled a total of 34 MS subjects, followed for three months to assess safety. The findings were extremely positive: “RTL1000 injection was safe and well-tolerated at a dose of <60 mg, with no evidence of global immunosuppression,” Vandenbark reveals. These findings, supported by new preclinical data, have provided the rationale for an upcoming phase II trial to prevent brain atrophy, reduce inflammation and promote CNS recovery in human secondary progressive MS patients.

There was an additional important outcome from the study, which helped the team to identify the key target of the RTLs: CD74 – the major receptor for macrophage migration inhibitory factor (MIF). Indeed, by studying RTLs in disease models, they discovered that its activity results not only from the selective modulation of specific T-cells, but mainly from its ability to block MIF binding to CD74 on APCs. “Although both functions are present, we believe that the high affinity binding of RTLs to CD74 on APCs far outweighs their low affinity binding to the TCR,” states Vandenbark.

MIF is an important molecule in MS. It is thought to contribute to pathogenesis by recruiting leukocytes to the CNS, inhibiting the apoptosis of self-destructive monocytes and T-cells and increasing the secretion of pro-inflammatory cytokines. RTL1000 works because it contains the DRα1 domain that binds strongly to CD74 and blocks MIF’s inflammatory effects. Vandenbark’s team plans to begin a phase II trial for the use of RTL1000 in MS this year.

**CLINICAL IMPACT**
In preclinical studies, RTL1000 was highly effective in reversing CNS inflammation and paralysis, preventing microglial activation, protecting axons from damage and promoting re-myelination, results that were virtually unprecedented. A drug with therapeutic, neuroprotective and neurorestorative effects, RTL1000 has huge potential.

Spurred on by their progress to date, the researchers want to improve on the RTL developed so far by assessing the potential of DRα1/peptide constructs as a next generation therapy. All humans have the same DRα allele, and are therefore tolerant to DRα1 injections. “This would allow treatment of all subjects without the need for tissue typing to match the DR2 allele used in RTLs,” Vandenbark explains.

Using the same simple structure – class II MHC alleles coupled to disease-inducing peptides – Vandenbark is aiming to treat many other immunologically-based diseases. “We want to define how the RTL technology is reducing demyelination and axonal damage and promoting neuroregeneration. This could be very useful for the treatment of neurodegenerative conditions,” he concludes.

**A PLATFORM WITH POTENTIAL**
As well as MS, RTLs have shown efficacy in models of:

- Stroke
- Methamphetamine abuse
- Arthritis
- Diabetes
- Optic neuritis
- Liveritis
- Macular degeneration
- Coeliac disease

Future possibilities include:

- Traumatic brain injury
- Alcohol abuse
- Alzheimer’s disease