OLLI 492: Human Immune System

Session 5: April 6th Summary and Observations

Chapter 4: A Multibillion-Dollar Blockbuster

In this chapter, Davis takes us back in history once again, focusing on the work of Sir Marc Feldmann as he sought to understand autoimmune diseases. The impetus for his choice arose from his "dissatisfaction" with the way the study of the immune system was being conducted as he began his career, with its focus on individual cells. Instead, Feldmann "… wanted to know what's happening in **the body, across the system**, not just what's going on inside a single type of immune cell. His thoughts turned to the way that different immune cells talk to one another."

"To study this, he set up a flask containing two glass tubes, one inside the other, with a porous membrane at the ends of both tubes, and filled the flask with culture broth. The broth could flow freely through the membrane but larger particles, such as cells, could not. With this set-up he could put different types of immune cells in the inner and outer tubes and keep them separate, while allowing them to bathe in the same culture broth. He set up several of these flasks and by comparing what happened in them with ones without separate tubes so that cells moved around and interacted with each other freely, he could assess which kinds of immune reactions required direct contact between cells and which could be triggered by secretions from cells into the liquid. A handful of other scientists were doing similar experiments around the globe, and even though they had almost no understanding about what was in the liquid specifically, they were essentially all studying the effects of **cytokines**."

This gave Feldmann a gross picture of what was going on. Davis notes that at the time, it would have been an "almost hopeless task" to get a coherent picture of what cytokines did "… because there was no way of isolating different cytokines and therefore establishing if the various effects on each type of immune cell were caused by one or several of them. It was only after the cytokine genes had been isolated, allowing the different cytokine proteins to be produced individually, that the effects of each could be studied systematically. This showed that each cytokine had multiple diverse activities…"

Unfortunately, Davis notes that "… with the tools in hand to properly dissect the cytokine world, the excitement of a gold rush ensued, electrifying (or intoxicating, depending how you view such things) some of the scientists involved with the prospect of money and fame."

As an example of this mentality, Davis next gives us a lengthy description of a dustup that occurred between scientists in "October 1984 at the fourth cytokine workshop" in the Bavarian Alps. "Philip Auron from Charles Dinarello's lab at Massachusetts Institute of Technology (MIT) announced that his team had isolated the gene for one of the forms of the cytokine named IL-1." At the end of his presentation, a scientist in the audience shouted that what Auron had shown was not the IL-1 gene. "The heckler was Christopher Henney, who, in 1981, had co-founded the biotech company Immunex, based in Seattle."

I won't go into the details of all the disputes that went on between Immunex and the small biotech company, Cistron, that was working with the MIT team. It took twelve years to settle the legal cases this dispute spawned, with little positive effect on the study of autoimmunity.

Meanwhile, Feldmann "… mused whether immune cells might activate each other through their cytokine secretions to such an extent that the activation becomes **self-perpetuating**, creating a vicious circle that overstimulates the immune system and causes it to harm the body. This

was a powerful new idea. Though he had little proof of it being true he put it out there nonetheless, he recalls, in the 'slightly overconfident mode of the young'." Davis notes that this sort of publication is unlikely to occur in today's publishing environment, but "… ideas can sometimes move us forward even in the absence of evidence, and the most important implication of this one, from a medical point of view at least, was that **blocking a cytokine** might stop immune cells from driving each other on and thus prevent autoimmune disease."

"Feldmann decided to focus on one autoimmune disease in particular, rheumatoid arthritis, a long-term inflammation in joints which causes pain, stiffness and sometimes disability…. We don't understand precisely how the problem starts, which probably varies in different people, but the symptoms come about because immune cells accumulate in joints and, over time, cause the destruction of cartilage and bone." While there is only a small influence of genetics in the cause of the disease, "… there are many **non-genetic factors** involved, which we don't understand very well…. At the time Feldmann set his mind to working on rheumatoid arthritis, the feeling among experts was that this was a very complicated disease, with many factors involved, so no simple treatment, certainly not a drug which targeted one particular molecule, would be likely to help"

After Feldmann moved to London to continue his research on rheumatoid arthritis, he partnered with "… the clinician Sir Ravinder 'Tiny' Maini."

This partnership would prove to be very beneficial. "Choosing to tackle rheumatoid arthritis as opposed to other autoimmune diseases was important because the relevant human tissue was accessible to study…. Studying cells and fluid isolated from patients' joints is what set Feldmann and Maini apart from most other researchers, and it's what got them on the right path towards finding a way to tackle the disease. They discovered that many cytokines were present but that one – with the unwieldy name '**tumour necrosis factor**' (sometimes called 'tumour necrosis factor alpha' and usually abbreviated to TNF) – was especially abundant."

Davis notes: "… every cytokine has a multitude of activities and TNF's ability, at high doses, to kill tumours was not what interested Feldmann and Maini. Rather, they wanted to test what would happen if they blocked the activity of TNF in the inflamed joints of arthritis patients. To do so, they needed an **anti-cytokine** – which is something that can be produced in the form of an **antibody**."

Davis now gives us a lengthy and detailed description of antibodies and how they work: "Antibodies are secreted by the white blood cells known as B cells and are our body's 'magic bullets'… They are soluble protein molecules that stick to and neutralise all kinds of germs and other potentially dangerous molecules. Each individual B cell produces an antibody with a uniquely shaped tip, the part of the antibody that sticks to its target molecule, called an antigen, which might be, for example, something on the outer coat of a bacteria or virus. However, antibodies are not designed to bind to germs per se. The shape of each antibody's tip is created almost randomly by a process of chopping up and rearranging the genes that create the antibody, a remarkable process in its own right. B cells which happen to have made an antibody that could stick to healthy cells and tissues are killed off (or inactivated) so that the only B cells allowed in the bloodstream are the ones that make antibodies that stick to something not normally found in the body. This is the process we first met in Chapter One, and is how these cells are able to distinguish self, components of your body, from non-self, anything that's not part of you."

Furthermore, "…. every B cell also has a version of its own antibody tethered to its surface (the B cell receptor we also met in Chapter One), so that the cell can tell when there is something in

the body that its antibody could lock onto. When a B cell does have the right antibody to lock onto something alien and troublesome, the B cell multiplies so that its useful antibody is produced in bulk, ready to neutralise the intruding molecule or germ. With around 10 billion B cells in the average person's immune system, each of us has the ability to make around 10 billion differently shaped antibodies, each of which is able to recognise something that hasn't been in the body before, ensuring that antibodies can be produced against virtually any structure alien to the body. This is essential if our immune defence is to tackle germs which the body hasn't seen previously – including germs which have never even existed before. Crucially for Feldmann's and Maini's purposes, it also means that any animal could make antibodies against a protein found in any other animal. Therefore a mouse immunised with the cytokine TNF could produce antibodies that would lock onto the human cytokine and stop it working – an anti-cytokine."

"Precisely such an antibody was made by scientist Jan Vilček at the New York University Medical School." Davis next gives us a lengthy description of the amazing life story of Vilček, … quite astonishing.

Returning to the topic at hand, Davis continues: "To make the antibody, Vilček first had to obtain a sample of the human cytokine, TNF, to be injected into mice. In late 1985, the company Genentech had isolated the gene for TNF and obtained significant quantities of the protein by expressing the gene in bacteria. Vilček was able to obtain samples of it in 1988 because he was collaborating with them on another project…. First, Vilček immunised mice with Genentech's TNF protein and, after a few days, isolated B cells from their spleens, knowing that many of these B cells would be producing antibodies against TNF. …. [Since B cells can't survive for long outside the body] Vilček used a trick to keep them alive – the Nobel Prize-winning trick that Milstein and Köhler had hit upon – which was to fuse the B cells with myeloma tumour cells and create new cells, called **hybridomas**, which retain the growth traits of a tumour with the antibody-producing abilities of the original B cell. In effect, this creates immortal versions of the mouse B cells. Vilček then isolated each single hybridoma cell…."

"This type of antibody is called a monoclonal antibody as it derives from a single B cell. The process can be used to create a protein shaped to lock onto any molecule of our choosing."

Davis notes that "Vilček had a long-standing agreement with the then fledgling company Centocor to develop commercial applications from the antibodies made in his lab."While Vilček focused on the science of producing the anti-TNF antibody, others were focused on the medical applications of it. Davis cites Bruce Beutler who "… worked, earlier in his career, with Anthony Cerami at the Rockefeller University Hospital, and discovered the mouse version of TNF. In 1985, he found that TNF was one of the cytokines produced in mice during sepsis, a disease caused by an immune response going into overdrive, usually because of a bacterial infection. Importantly, Beutler and Cerami found that blocking TNF could protect mice from the symptoms of sepsis."

"In humans, sepsis (called septic shock when symptoms include a drop in blood pressure) can kill patients in a matter of hours…" Centocor was also focused on treatments for sepsis, and inspired by Beutler and Cerami's work, "… they wanted to try treating sepsis in humans by blocking TNF."

Davis notes that Centocor "… couldn't use Vilček's anti-TNF antibody in people straight away. Since the antibody was made in a mouse, it had to be modified to more closely match antibodies naturally made in humans. Otherwise, the antibody itself would be seen as something alien in the human body and could trigger an immune reaction. To avoid this,

segments of genes for the mouse antibody were combined with human genes to create a new half-mouse half-human antibody."

Centocor tested this **chimera** on humans with sepsis in 1991. There was no therapeutic benefit. "What worked in mice didn't work as well in humans; a common theme in medical research." Coincidently, "in early 1991, Feldmann visited Centocor to present his case for trying the antibody in patients with rheumatoid arthritis."

"… Feldmann had some evidence to support the idea that TNF was important in rheumatoid arthritis and that blocking its activity might help. Maini's team had found that the cytokine was present in the right place at the right time for being involved in the symptoms of the disease. In Feldmann's team, Fionula Brennan (who sadly died young from breast cancer in 2012) had looked at what happened when an anti-TNF antibody was added to cells taken from the diseased joint of patients. The result was a eureka moment. Brennan discovered that when TNF was blocked, other cytokines stopped being produced by the cells. She repeated the experiment seven times to be absolutely sure. This implied that TNF was at the top of a cascade of events, or the hub of a network, which led to the other inflammatory cytokines being produced."

Another of Feldmann's team tested the idea in mice by immunizing them with collagen, causing an immune reaction. "The afflicted mice were then given an injection of anti-TNF antibody and, at high doses, the inflammation was reduced and cartilage at the animal's joints was spared from damage. This showed that mice could be relieved of the symptoms of arthritis by an injection of anti-TNF antibody."

Centocor remained skeptical this would work in humans. But, "James 'Jim' Woody, who had done research for his PhD under Feldmann's supervision in London, was now the chief scientific officer at Centocor." Woody successfully advocated for a small trial. Davis notes: "A personal connection is often what's needed to make things happen."

Feldmann and Maini began a small trial in April 1992 in London. There was immediate improvement: "… the reduction in swelling and tenderness in patients' joints was formally significant after two weeks." Many patients returned to their normal lives, pain free. Davis describes the improvements in several patients. "But unfortunately the benefits were short-lived. Everyone relapsed."

"… the antibody wasn't a cure; but it could relieve symptoms. This meant that the next logical step was to test the benefits of blocking the cytokine repeatedly. Feldmann and Maini obtained ethical permission to re-treat some of the patients, and again, all those tested improved. Still, the results were anecdotal, with no controls…" A proper clinical trial was needed.

"The results of the first formal trial were unequivocal; anti-TNF antibody improved the health of rheumatoid arthritis patients. Detailed analysis of what was happening in patients' blood revealed that the antibody was working just as Feldmann and Maini predicted: blocking this one cytokine reduced the production of other inflammatory cytokines, and biopsies showed that fewer immune cells were entering the diseased joints."

Centocor became interested: "For the next clinical trial – a full-scale comparison of anti-TNF antibody with existing treatments, a so-called phase III trial – Centocor was keen to get their antibody approved as a medicine as fast as possible, so fewer samples were taken and, Feldmann says, the emphasis on detailed analysis was lost."

Nevertheless, “The phase III trials proved that anti-TNF antibody was an effective therapy and was better than other treatments available at the time…. This led directly to what is commonly prescribed to patients today: anti-TNF antibody usually taken with another drug, methotrexate, which has many effects in the body including, it turns out, dampening T cell immune responses. This was an early example of medicines being used in combination to treat a disease, something that’s far more common today. Poly-pharmacy, Feldmann calls it.”

“Centocor’s ambition to treat sepsis was never fulfilled; the disease remains notoriously difficult to tackle, likely because a storm of inflammation builds up so rapidly in the body that it’s especially hard to control.”

Davis recounts the commercial effects of both the failure of the sepsis treatment and the success of the rheumatoid arthritis treatment. Feldmann regretted that another British discovery was commercialized in the U.S. He notes that several other companies became interested in Feldmann’s treatment. “Beutler helped create one of the alternatives: a soluble protein version of the cytokine’s natural receptor. Effectively this acts as a decoy receptor to prevent the cytokine’s engagement with its real receptor on immune cells. Clinical development of Beutler’s drug, led by Immunex, began two years behind Centocor’s but raced ahead to such an extent that in November 1998 it became, in fact, the first anti-TNF medicine approved to treat rheumatoid arthritis in the US, marketed as Enbrel.” He also notes that several of these drugs became “blockbusters” - sales of >$1 billion dollars per annum.

But Davis notes that this therapy found uses in other conditions: “Blocking this cytokine helps stop inflammation in many situations where it is a problem: in the digestive system, as happens in Crohn’s disease and colitis; in skin, as happens in psoriasis; and in the joints of the spine in ankylosing spondylitis.”

“This success did not come about in any easy or linear way; it took a multitude of small steps for Centocor to make the part-mouse part-human antibody, tested by Feldmann and Maini, based on the antibody Vilček made first. Triumph came from imagination and hard work, but also from a web of coincidences, chance events and serendipities”

Davis also notes that many of the participants in this episode were awarded prestigious prizes for their work. And that others pursued other cytokines as therapies for inflammatory diseases (he cites IL-6 being effective against rheumatoid arthritis). He sums up: “It takes a community to explore all the possibilities.”

He finally notes: “Indeed, unlike vaccination – discovered long before anybody had any detailed understanding of how it worked – anti-TNF therapy emerged directly from understanding the molecules and cells that make up our immune system; knowledge that was generated by thousands of scientists. We tell the stories of individuals – and perhaps ego propels scientists into action – but no scientist is an island. At some level, this therapy was achieved by a collective scientific mission to understand immunity. Maini is especially proud of this fact; that his work helped show how the detailed molecular science of immunology can be harnessed for medicine.”

“The discovery of anti-TNF therapy was a watershed moment because it introduced a new way to combat disease – manipulating the immune system rather than directly fighting germs with, say, antibiotics, in a way that’s very different to vaccination.” Davis notes the increased interest in controlling cytokines as a potential therapy for a wide range of conditions and diseases.

Davis, however, introduces a note of caution: "There are at least three significant problems with anti-TNF therapy. First, blocking this part of the immune system inevitably weakens our defence against infections." He cites the potential for latent tuberculosis to flare up as a result of the compromised immune system.

"A second problem with anti-TNF therapy is that a significant fraction of patients do not benefit from it: as many as four in ten rheumatoid arthritis patients show little improvement. Combinations of drugs can improve response rates, but unfortunately, we currently have no way of knowing in advance who will respond and who won't." And: "The third problem is that blocking TNF is an effective treatment, but not a cure. The quest for a cure continues."

"Feldmann's and Maini's research also had far-reaching consequences because of the type of medicine they used – an antibody. At the time, the potential for antibodies to be used as medicines was not widely recognised because they were – and still are – very expensive to produce." Davis describes just how complex the process of producing the antibody is.

"Ever since Milstein and Köhler learnt how to make antibodies à la carte, it seemed that there had to be a role for antibodies in medicine. But in practice, for the nearly two decades it took for anti-TNF to be developed, the pursuit felt like chasing a rainbow."

Davis continues with another example: "One of the most important antibodies developed subsequently is rituximab. Instead of blocking a cytokine, this antibody directly targets immune cells, specifically B cells. When it locks onto a protein molecule on the surface of a B cell, that particular B cell is destroyed in one of three ways. First, the antibody itself can cause the B cell to self-destruct. Billions of our body's cells die this way every day to allow for a healthy turnover of cells in the body; rituximab can simply trigger this same programme of cell death. A second way that the antibody kills B cells is that, while its front end is tethered to a B cell, its back end attracts factors in the blood which then kill the B cell. Alternatively, its back end can be recognised by the immune system's Natural Killer cells, which flatten up against the B cell and kill it. Again, these last two processes happen as part of our normal immune defence; antibodies usually lock onto germs or infected cells, things which warrant attack, in this way. Rituximab essentially causes a person's own B cells to be detected by the immune system as something to be eliminated."

"The loss of B cells in the body that results from this antibody can in turn dampen inflammation in a patient's joints, so rituximab is prescribed as an alternative medicine for those rheumatoid arthritis patients who do not benefit from anti-TNF therapy. However, it was first approved not for the relief of rheumatoid arthritis but in 1997 to treat cancer. It has since been used by over 750,000 cancer patients. At a glance, it seems quite unlikely that a cancer drug could help with rheumatoid arthritis; these maladies have little in common. But an antibody which kills B cells is useful for the types of cancer – chronic lymphocytic leukaemia and non-Hodgkin lymphoma – where it is a B cell that has lost control and become malignant." Davis notes that the WHO lists rituximab as one of "the world's most essential medicines."

Davis next delves into what he calls the "edge of knowledge." He relates the efforts to understand just how rituximab works, and how other antibodies could be designed to be just as effective. But he notes that all these efforts involve activity in a lab petri dish. "It is alas impossible to watch whether or not antibodies trigger these events inside patients. This was, after all, Feldmann's point at the outset: we need to know what happens inside the body, where the entire system is at work, not just in isolated cells in a lab dish."

"The success of the anti-TNF antibody, and rituximab, began a fashion for seeking more antibody-based medicines, but in 2006 momentum was lost. Controversy erupted when a

clinical trial testing a different antibody drug, named TGN1412, went horribly wrong." The drug uses a novel activation method for T cells, aimed at fighting cancer.

"In the patients, the drug activated T cells to such a high degree that they began to attack the body's healthy cells and tissue. The overly active immune cells also released cytokines at such high levels that they became toxic to the body. What happened to the patients in the trial is somewhat like what can happen in sepsis, an overreaction of the immune system caused by an acute bacterial infection." Davis describes the patients awful symptoms, but notes: "Thankfully, nobody died, but the clinical trial was a tragedy."

Davis notes that the fallout of this trial was huge and led to major changes in how human trials are conducted. "The important lesson for science was finding out – all too dramatically – that tinkering with our immune system is like trying to harness nuclear power: there is great potential but a mistake can be catastrophic."

Davis sums up: "… the discovery of anti-TNF therapy showed us that a detailed knowledge of immunity pays off, not only because it reveals a hidden beauty in how the human body works but because this is an area of science that leads to new medicines. Still, the road to each new medicine is not a highway; it is a narrow lane, uncharted on the satnav [GPS?] and full of blind corners. Driving fast is unsafe. We must map more of the immune system to understand how and why its activity varies, to understand the boundaries it operates safely within and, crucially, how it connects with other body systems."