OLLI 492: Human Immune System

Session 2: March 16th **Summary and Observations**

Overview

The author, Daniel M. Davis, presents his objectives for the book in the Overview. I won't go into much detail about it here, but you should read it; it gives you a good sense of the author's style and approach. But I do want to draw your attention to a few issues.

A constant theme of the book is how recent the discoveries that deepened and expanded our understanding of the immune system are, and how complex the system is. He notes: "This crucial realisation, that an immune response can't be triggered by just anything alien to the human body, came only **as recently as 1989**, and it would take many more years before a deeper understanding emerged…. the world of immunity has opened up to reveal what it really is: not a simple circuit involving a few types of immune cells but **a multilayered, dynamic lattice of interlocking subsystems**, one of the most complex and important frontiers of scientific enquiry we know of."

Another theme of the book is how much still is unknown and remains to be explained., and how difficult it is to control the immune system "**Mysteries remain** but already these discoveries challenge the simple view we once held about how our bodies fight disease – and what it takes to be healthy. Even though it's correct – very roughly – that the immune system targets what's not part of you, it has become apparent that layer upon layer of **biological checks and balances**, run by countless cells and molecules, regulate the process."

In addition, the author frequently refers to the changeability of the immune system, that it changes over short timespans, and that it weakens as we age, complicating the development of therapies using the immune system. "We have learnt that part of the problem is that the elderly have fewer of some types of immune cells circulating in their blood. Another is that immune cells in the elderly are worse at detecting disease."

And lastly, he constantly urges us to take a holistic approach to understanding the immune system; we need to view the system in the context of the whole organism. "*The Beautiful Cure* is about **the bigger picture**: how and why the activity of our immune system varies, how it is regulated and directed, all of its component parts – t**he whole shebang**."

Chapter 1: Dirty Little Secrets

Davis begins this chapter with an introduction to the immunologist, Charles Janeway, and the puzzle that inspired his great insight and that led to the discovery of the innate immune system.

The puzzle grew out of the standard view of how vaccines work: "So – the dogma goes – vaccines work by exposing you to a dead or harmless version of a germ. By provoking your immune system to build up defences against it, it prepares you to respond rapidly if you encounter the same germ again. This works because the particular immune cells that are activated by a particular germ multiply and persist in the body for a long time, long after the germ has been eradicated, meaning they are ready for action if they encounter the same germ again."

But: "The 'dirty little secret' is that vaccines only work well when so-called '**adjuvants'** are added. Adjuvants (from the Latin word *adiuvare* meaning 'to help') are chemicals, such as aluminium hydroxide, which, as discovered by chance, help vaccines be effective."

The need for adjuvants "…revealed a crack in our basic understanding, because no one could actually explain why adjuvants did this. …Janeway was determined to understand precisely **why adjuvant was necessary**. In doing so, he uncovered a whole new way of thinking about how the human immune system *really* works."

The author next proceeds to give us an abbreviated account of the history of vaccinations.

The author concludes this history by noting: "By the time of Janeway's epiphany in 1989, the consensus view was that the presence of a germ in the body triggers an immune reaction because the body is primed to detect **molecules** that it has not encountered before; in other words, that the immune system works by reacting against **molecules that are non-self** – not from the body. After exposure to molecules alien to the body, the immune system is poised to react rapidly if the same non-self molecules are encountered again."

Davis then recounts two experiments, performed in the 1920s, that undercut this consensus view, performed separately by two different researchers. "They each discovered that a **protein molecule** made by the bacteria which cause diphtheria – diphtheria toxin – could be inactivated by heat and a small amount of the chemical formalin. Potentially this meant it might be used as a safe vaccine against the disease. To their surprise, however, when the inactivated protein molecule was injected into animals, the immunity it produced was only short-lived."

"Janeway reasoned that protein from the bacteria was non-self – not part of the human body – and so, according to the consensus view of the 1980s, there was no explanation for why it would not work well as a vaccine. How come the pus from cowpox blisters worked well as a vaccine, Janeway wondered, whereas protein molecules such as diphtheria toxin, which had been isolated from germs, did not?"

One of these experimenters, Alexander Glenny in London, was "…seeking a way to make diphtheria toxin work as a vaccine. Eventually, in 1926, Glenny's team found that when diphtheria protein was purified by a chemical process that involved combining it with aluminium salts, it became an effective vaccine. Glenny's explanation was that the aluminium salts helped the diphtheria toxin stay in the body long enough for an immune reaction to develop, but no one knew of any process which could explain how or why this might be."

The need for an adjuvant to make a vaccine effective led to Janeway's insight: "In January 1989, Janeway and his wife, fellow immunologist Kim Bottomly,…realised that they could not easily explain how an immune response starts: what exactly was the trigger? …For the next few months, he mulled over the problem – how does an immune reaction start? – as well as the question of how adjuvants work, and it was by **thinking about the two problems together** that he had a revelatory idea."

It was known that a large molecule found in the outer coat of bacteria, lipopolysaccharide (LPS), was an especially effective adjuvant. "What if, Janeway reasoned, the presence of something that has never been in your body before was not the sole indication that an immune reaction should occur? What if there has to be something else – a second signal – that's needed to kick off an immune reaction, a second signal that can be provided by an adjuvant, which might in turn **replicate** the presence of actual germs?"

Janeway presented this idea "…in a now-famous paper entitled 'Approaching the asymptote? Evolution and revolution in immunology'…" In this paper, "He suggested that distinguishing between self and non-self was not enough: the immune system has to be able to tell when something is likely to be a threat to the body **before an immune reaction takes place**, and that therefore the immune system must, he reasoned, be able to detect **telltale signs of actual germs** or infected cells. He predicted that there had to be a whole part of our immune system, yet to be identified, with this very purpose, and he even predicted a way it could work."

Up to this point, the author has been using "immune reaction" to refer to the activation of white blood cells, namely T Cells and B Cells; the surface of these cells have receptors, T cell receptor and B cell receptor. "These receptors come from the class of biological molecules known as proteins, which are long strings of atoms that fold up into elaborate shapes well adapted for a specific task in the body. In general, proteins bind or join with other molecules, including other proteins, to complete their tasks, and the precise shape of a protein dictates which types of other molecules it is able to connect with, in the same way that two jigsaw pieces interlock by having complementary shapes. The receptor on each individual T cell or B cell has a slightly different shape, allowing it to interlock with a different foreign molecule. It reaches out from the immune cell's surface into its surroundings, and if it connects with something that hasn't been in your body before, it 'switches on' the immune cell, which then kills the germ or infected cell directly, or summons other immune cells to help. Crucially, the activated immune cell also multiplies, populating your body with more cells that have the same usefully shaped receptor. Some of these cells stay in the body for a long time, which is what gives the immune system a *memory* for germs that have been encountered before – which is, of course, at the heart of how vaccination works."

The author goes on to describe how the body protects itself from harm caused by T and B cells: "The way in which the body ensures they only latch onto germs is one of the greatest wonders of the immune system, and works as follows. Each T cell and B cell acquires its receptor while developing in bone marrow. A **shuffling of genes** as the cell develops gives each cell a uniquely shaped receptor. But before entering the bloodstream, each individual T cell and B cell is tested in case its receptor is able to bind to healthy cells. If it is, then that particular T cell or B cell is killed off, because it would be dangerous to have such an immune cell in the body. In this way, only T cells and B cells that won't attack healthy cells are allowed to defend the body, and by the same logic, if a receptor on a T cell or B cell does bind to something, that something must be a molecule that hasn't been in your body before. In formal language, this is how the immune system is able to distinguish self, the components of your body, from non-self, anything that's not part of you."

Janeway was not satisfied with this notion of an immune response; he "…predicted that there must be receptors (which he called **pattern-recognition receptors**) that are not randomly generated and then selected, but rather have fixed shapes that interlock specifically with germs or infected cells (or rather with molecular patterns that are found only on germs or infected cells). Because this appeared a much simpler way for immune cells to detect germs compared with the elaborate process of making immune cells with randomly shaped receptors and then killing off those which might react against healthy cells, Janeway suggested that **receptors with fixed shapes** probably evolved first to defend against disease, and only later, when life on earth became more complex, did a more elaborate immune system develop, which then included T cells and B cells."

"The simpler system of fixed pattern-recognition receptors that Janeway predicted forms part of the system often called **innate** immunity, by contrast with the aspect of our immune defence that accounts for its memory of past infections, which is called **adaptive** immunity. "

"Before Janeway, the raison d'être of the immune system was to react against things that have never been in your body before. But Janeway said that the immune system must respond to things that haven't been in your body before – and are from germs."

Davis notes that there is "…a crucial problem with Janeway's idea: each time a germ reproduces, it acquires random changes in its genes – mutations – and through these changes it seems likely if not inevitable that some will lose the molecular signature detected by the immune system. In other words, in the whole population of viruses or bacteria, some of them will by chance – because there are so many – have acquired a genetic change which alters the part of the germ which the pattern-recognition receptor was designed to lock onto. Microbes lacking the 'molecular pattern' would escape detection by the immune system and multiply readily."

"Janeway realised this and so he predicted that 'the pattern recognised should be the product of a complex and critical [process] in the microorganism'. In other words, the telltale structure of a germ would have to be **something so critical** to its lifecycle that it would be exceptionally difficult, if not impossible, for the germ to alter it. "

That something so critical to the life-cycle of a bacterium involves cell division: "Every time one bacterium divides, it needs to build a cell wall or envelope to encapsulate its two daughter cells. Importantly, the process is so complex that bacteria can't easily change it. Penicillin works by interfering with the last stage of the process. As a result, there isn't any simple genetic mutation that would allow bacteria to escape penicillin's effects. True, bacteria can become resistant to the antibiotic by making their cell walls with a very different process, but it's not easy, which is why penicillin remains effective against a huge number of microbes: it locks onto bacterial protein molecules involved in an essential and complicated process."

However, the author notes that Janeway's ideas were ignored by other immunologists, all but forgotten, until: "In autumn 1992, a student at Moscow University, Ruslan Medzhitov, read Janeway's paper and it changed his life."

Davis gives an account of Medzhitov's peripatetic journey to Janeway's lab. "On 2 January 1994, Medzhitov finally met Janeway face to face. Both were big-picture thinkers, passionate about ideas, and a lifelong partnership and friendship began. The duo's immediate mission was to find out if human immune cells really did have **'pattern-recognition receptors**' able to detect telltale signs of germs."

Earlier work on insect immune systems, done in the 1960's, pointed the way. The research was begun by Pierre Joly who "…was joined in his lab by a twenty-three-year-old PhD student named Jules Hoffmann, keen to study insects because his father was an entomologist. Hoffmann set out to understand the insect immunity that Joly had observed and began working with grasshoppers."

After Joly retired and Hoffmann took over the lab, he switched to studying drosophila (the fruit fly). "His team then set about answering two crucial questions. What kinds of molecules had endowed the fly's blood with an ability to kill germs? And second, which genes controlled the fly's immune response? The first question turned out to be quite easy to answer. Specific kinds of molecules (short pieces of protein, known as peptides) had been identified in silk moths as being antibacterial, and Hoffmann's team found similar molecules in their flies, with different ones being able to kill different kinds of germs."

The switch to drosophila proved crucial in answering the second question; drosophila's genome was the subject of intense investigation and sequencing efforts. These efforts provided Hoffmann with vital clues. "One clue was that an insect gene named **toll** – from the German word *toll*, meaning 'great' – which was important in the development of the fruit fly embryo turned out to be similar to a human gene (called the IL-1 receptor) already known to play a role in immunity. Also, certain genes present in both flies and humans (known as NF-kappa-B transcription factors) had recently been discovered to be important for human immune responses."

Experiments performed by Bruno Lemaitre in Hoffmann's lab from 1993 to 1995 showed that "…flies were dependent on the toll gene to be able to clear fungal infection. This was a spectacular discovery – firmly establishing that genes involved in the embryonic development of the fly were also part of its immune system – and was immediately recognised as such."

Meanwhile, Medzhitov in Janeway's lab, ramped up his work on the human equivalent of toll genes. "… he discovered that it [the human toll-like gene] could switch on the activity of other genes (specifically NF-kappa-B transcription factors) known to be involved in immune responses. Put together, the implication of these discoveries was profound: they showed that life forms as different as insects and humans share a genetic heritage for fighting disease."

"Other research teams then uncovered many more genes, in mice as well as humans, like the insect toll. Collectively, they are called toll-like receptor (TLR) genes – named for being a set of genes where each encodes for a receptor protein similar to insect toll – and there are ten in humans. As work progressed each gene was given a number. Medzhitov's original human toll is now called TLR4. Experiments with mutant mice showed that these different toll genes were essential for immune reactions to all kinds of bacteria and viruses. Even so, while it was clear toll genes were somehow important for immunity, nobody really knew how they worked. Not until 5 September **1998**."

Davis now turns his attention to the research that led to that important discovery. Bruce Beutler, working out of his lab in Dalles, was obsessed with finding the genes involved in immune reactions, i.e., "… finding out which gene was crucial for an immune reaction to occur in mice that had been exposed to lipopolysaccharide, or LPS – the chemical normally found in the outer coating of bacteria, which had been shown to be an especially potent adjuvant. The problem was widely known as important because the gene involved would likely give a big clue as to how this bacterial molecule was sensed by the immune system"

That obsession finally paid of: "On the evening of 5 September, he [Beutler] was filled with joy when an analysis on his computer screen indicated that the crucial gene for detecting the bacterial molecule LPS in mice was very similar to Hoffmann's insect toll gene and Medzhitov's human gene, TLR4."

The author sums up: "Finally the pieces came together to reveal the big picture: the TLR4 gene encodes for a protein molecule that is able to interlock with a component from the outer wall of bacteria (LPS). In other words, the TLR4 gene encodes for a **pattern-recognition receptor**, the very type of molecule that Janeway had predicted existed – one of the eyes of the immune system, as Beutler puts it – giving immune cells with this receptor protein protruding from their surface an innate ability to lock onto bacteria. When TLR4 locks onto the bacterial molecule LPS, this signifies that there is something in the body that may very well require an immune response."

Davis notes that other teams were working on the same problem, and came to the same conclusion. But Beutler published first in December 1998. The result: "Thirteen years later, on 3 October 2011," he, Jules Hoffmann, and the Canadian immunologist Ralph Steadman shared the Nobel Prize in Physiology or Medicine.

The author notes that "… 24 eminent immunologists" protested in a letter to *Nature* that the Nobel committee should have recognized the "seminal contributions" of Janeway and Medzhitov. But Janeway had died in April 2003 from lymphoma; the Nobel Committee rules would have excluded Janeway from the award. "His obituary in *Nature* said that while 'most scientists only dream of contributing to a paradigm shift – Janeway personally initiated one'."

Davis spends sometime on the fallout of this award, describing the rivalry, the bad blood, among the four researchers, between Beutler, Hoffmann, Lemaitre, and Medzhitov. I won't go into any detail about this here; you can read it for yourselves.

The author next sums up the importance of this research: "This watershed moment in our understanding of the human body has led to more than 30,000 scientific papers being published on toll-like receptors in the immune system in ever-increasing detail. The immediate next step was to find out what kind of germ each numbered receptor is able to see. While TLR4 locks onto a molecule in the outer wall of bacteria (LPS), TLRs 5 and 10 have been shown to lock onto molecules found in parasites, TLRs 3, 7 and 8 detect some types of virus, and so on. The flood of research that followed also revealed that the toll-like receptors are just one kind of pattern-recognition receptor; there are many others, with cumbersome names such as nucleotide oligomerisation receptors, C-type lectin receptors and retinoic acid-inducible gene-1 (RIG-1)-like receptors."

"Not only is each pattern-recognition receptor able to detect a different kind of germ, each is also positioned differently in the body – strategically located in a place where that germ might be found." Davis goes on to give examples of this distribution.

He continues with his assessment of the importance of the work: "Before these discoveries it was thought that innate immunity was merely a broad-brush defence, in the same way that skin, for example, could be thought of as a simple barrier against all kinds of germs entering the body. But when a multitude of different pattern-recognition receptors were discovered – each equipped to detect specific types of germs and switch on a response that is appropriate to the threat – it became clear that the innate immune system is **far more complex** than had been imagined. The innate immune system doesn't just detect the presence of germs but can recognise the type of germ present and direct the immune response accordingly."

"The system (or subsystem) uncovered here – innate immunity – forms our **first line of defence**, an immediate response to the presence of germs. An opportunistic fungal infection or a bacterium entering a cut or wound is often dealt with rapidly by our innate immune system. It is only when our innate immune response can't deal with an infection fully that the adaptive immune response – the action of T cells and B cells – becomes important, a few days after the body has been infected. Infections resolved within two or three days are usually down to germs being detected by pattern-recognition receptors and the appropriate reactions they trigger."

Davis also notes that "…it soon became clear that there were **important medical outcomes** from these discoveries in innate immunity; the most pertinent, back where it all started, was to do with vaccination."

The author notes that **it is still a mystery** how aluminum salts help make vaccines work, but "…what did become clear was that adjuvants are important because they switch on the innate

arm of our immune system. As a result, instead of using aluminium salts, adjuvants could be tailor-made to switch on the innate response using the molecules that had been identified as the specific targets of pattern-recognition receptors." Davis describes how this finding has changed the way pharmaceutical companies research and make vaccines.

Finally, Davis notes: "Other medical applications are likely. Beutler and others think it may be possible, in the near future, to help autoimmune diseases with new drugs that block the action of toll-like receptors."

The author concludes on a philosophical note: "What does it take to do something great? I once asked Medzhitov if he thought Janeway had a particular trait that was important for his ability to have predicted so much, so many years before everyone else. He replied confidently that many scientists have one big idea, which they stick with throughout their entire career. Janeway, however, like all creative people, had many ideas, and above all he was never afraid of being wrong."