OLLI 492: Human Immune System

Session 8: April 27th Summary and Observations

Chapter 7: The Guardian Cells

The focus of this chapter is on the discovery of regulatory T cells, the genes that affect their development and functioning, and the result of their failure to successfully modulate the immune response - autoimmune disease. Along the way Davis also explores the area which holds the greatest challenge for the immune system's capability to distinguish self from non-self, namely the gut and its microbiome.

He begins by noting how complex and challenging it is to distinguish self and non-self. "To achieve this simple-sounding mission – to discriminate between what requires a response and what doesn't, and to deliver the right type of response – the human body has invested heavily in a galaxy of cells, proteins and other components, to create a system as elaborate as anything else we know of in the universe. And **sometimes it fails**."

Davis goes on to restate the process by which T cells are tested to make sure they don't attack self cells. "But the process isn't perfect, errors happen and healthy cells and tissues can be destroyed without good reason. This is the problem that underlies **autoimmune disease**."

After describing how the immune response can go wrong and attack healthy cells, he points to **the fact that there still remain large gaps in our understanding of how the immune system works, and correlatively, how it can go wrong.** He notes: "One reason why autoimmunity has been so difficult to understand is that everything about it is so deeply counterintuitive."

Davis next gives us a concise history of how the concept of autoimmunity finally became accepted as real, citing the publication in 1964 of the conference papers of an international workshop. "Autoimmunity was one of the most important surprise discoveries of twentieth-century medicine."

One clue about the functioning of autoimmunity came from noting that "… the same person sometimes shows symptoms of more than one type of autoimmune disease…. The implication is that the underlying cause of autoimmune disease is not necessarily something that happens in any one particular organ, but something that happens to **the immune system in general**, a **weakening** of its ability to discriminate between healthy cells and harmful germs."

This fact stimulated the Japanese scientist Shimon Sakaguchi to study autoimmunity as a route to understand the immune system as a whole. His research involved the removal of the thymus from mice, which resulted in destruction of tissue and autoimmune diseases. This resulted from the missing thymus: "… immune cells (T cells specifically) capable of attacking healthy cells and tissues are normally killed off in the thymus. In animals that have had their thymus removed at a young age, self-reactive T cells were not destroyed, which led to autoimmune disease."

For his PhD research, Sakaguchi repeated this experiment. The years of effort invested in this experiment (1979 to 1981) resulted in "…a simple dramatic outcome: first, mice had their thymus removed so that, as before, they developed autoimmune disease. These mice were then given an inoculation of immune cells from a healthy mouse (of the same inbred strain) and amazingly, this stopped the autoimmune disease. Mice could be given a dose of immune cells either before or after their thymus was removed and, either way, the autoimmune disease

stopped. In other words, Sakaguchi had discovered a cure for autoimmune disease which would otherwise have been inevitable."

Davis notes the importance of this discovery: "… he showed that among the immune cells of a healthy mouse, there must be some that stop immune reactions and can **stop autoimmune disease**."

But as is often the case, this concept that some types of immune cells can stop an immune response had come up before. He notes that "… In the early 1970s, several research groups around the world found that the addition of some types of immune cell would suppress, not boost, a reaction. Richard Gershon, working at Yale University with his assistant Kazunari Kondo, published their observation of this in the British journal *Immunology.*"

"Gershon proposed that there had to be some T cells that behave differently to normal T cells and he coined the term '**suppressor T cells**' to describe those that could stop, rather than help, an immune response. A decade later, Sakaguchi's experiment helped vindicate Gershon's idea – and extended it, showing that suppressive immune cells could be especially important in preventing autoimmune disease."

Problems arose. There remained a great deal of skepticism about this finding; other interpretations of the results were offered. "The main obstacle that prevented problems such as this from being quashed was the inability of anyone being able to separate suppressor T cells from normal T cells. The methods available were simply too coarse. Recall how the discovery of dendritic cells, discussed in Chapter Two, was only widely accepted after the cells could be isolated and then shown to have properties above and beyond other types of immune cell. Without a way to identify and isolate suppressor T cells, it was hard to prove their existence, let alone understand how they might work. But that didn't stop scientists guessing."

Davis notes that alternate theories abounded in subsequent years, leading to what he terms a "Dark Age" in the study of the immune system. "Eventually, new methods allowed for greater rigour [in analyzing the immune system]– and suppressor T cells were caught up in the cull of ideas that ensued. One especially damaging episode was when, in 1983, a region of the genome thought to control the function of suppressor T cells was shown to lack any such gene. Belief in suppressor T cells collapsed…. Suppression became a dirty word… 'No realm of immunology has less credibility than that of suppressor T cells,' scientists wrote in 1992."

But a small band of researchers, including Sakaguchi, **persisted**. "Once again, it was new technology that moved things forward, as is so often the case. Tools were developed that could mark out different types of T cell with far more precision, tagging them according to the different molecules they had at their surface."

These advances led to more teams working on this issue. Davis describes the work that two independent U.S. teams conducted in 1993. "The key to the advance that both teams made was to separate mouse T cells into two types. One group of T cells – formally called naïve T cells – was composed of cells that were ready and able to mount a defence should their receptor prove compatible with a new threat but which had yet to encounter such a germ and be deployed. The second set of T cells was composed of those that had already been 'switched on' and used in the body. This second set included a hotchpotch of T cells with different jobs, including those T cells that remain after the infection has been cleared in order to provide stronger immunity should the same germ attack again, as well as suppressor T cells, activated by the body's own components. The researchers transferred each of the two groups of immune cells into a different set of mice, all of which had been **genetically engineered to**

lack their own T cells, so that the only T cells present in the mouse would be those infused into it."

"They found that in the first group, the naïve T cells, those which had never been switched on before, would, in the absence of suppressor T cells, switch on and attack the mouse's healthy tissue, so that **they developed an autoimmune inflammation** in their gut. This established, albeit in this unnatural situation, that normal T cells could attack healthy tissue and cause an autoimmune disease. If these same mice were then given a dose of the second group of T cells, **the autoimmune disease was stopped**. This fitted precisely with the idea that those T cells responsible for fighting germs were also capable of attacking the body, causing autoimmune disease, but that other T cells – the suppressor T cells – could prevent this. The fact that two US research teams published their results within months of each other immediately validated the discovery."

Meanwhile, Sakaguchi refined a method for identifying suppressor T cells. "Instead of grouping cells according to whether or not they had been switched on before, he found, in 1995, that suppressor T cells had especially high levels of a particular cytokine receptor protein at their surface. He used this information to remove this set of T cells from the mouse immune system. To do this, T cells were taken from one mouse and those with the particular receptor protein were killed off. The remaining T cells were then injected into a second mouse, which again had been engineered to lack its own T cells. This second mouse now suffered autoimmune disease. This meant that removing suppressor T cells from a mouse's immune system was sufficient to cause illness. This directly supported Sakaguchi's big idea: that an **abnormality in suppressor T cells** could be what underlies many different types of autoimmune disease."

One of the influential skeptics of suppressor T cells was Ethan Shevach at NIH. He read Sakaguchi's publication of his results and assigned Angela Thornton to reproduce the results "Thornton found everything Sakaguchi had done to be true. And with that, Shevach changed his mind about the existence – and vital importance – of suppressor T cells." Davis notes that this "tuned heads everywhere." And Shevach "… shifted the bulk of his lab's research efforts to studying these cells."

"But there was still a problem. All of the research so far had been conducted on animals, or with animal cells, and none of it had been shown to be true in humans. This was probably for the simple reason that so few labs were working on suppressor T cells, a result of there being such scepticism about their existence for so long. Eventually, in **2001** – three decades after the idea of suppressor T cells had first been suggested – six different teams identified human suppressor T cells all at once."

Davis tries to explain the persistence of the skepticism: "The chief problem was that methods weren't available to **isolate suppressor T cells** so that they could be studied in detail. But my own view is that another issue contributed to the error: scientists of the era had been too quick to judge. The **complexity** of the immune system means that we now know that we cannot expect every interpretation of every experiment to be correct."

"By the time human suppressor T cells were widely accepted to exist, the name 'suppressor T cell' had already been used as a synonym for bad science for over a decade. It had to be changed; a new name for a fresh start. From here on, these cells were to be called regulatory T cells, or Tregs (said as T-regs, like the dinosaur T-rex). After decades of observing the shadows of regulatory T cells, they were finally in the spotlight and accepted as a crucial part of our immune system: **guardians of the galaxy**."

There still remained unanswered questions about regulatory T cells. One was identifying the genes that controlled their activity.

Davis next recounts the history of the discovery of these genes. It begins with studies on the effects of radioactive fallout from nuclear weapons. "In response to the Manhattan Project's successful production of the world's first nuclear weapons, the Mammalian Genetics Laboratory was set up in 1947 at the Oak Ridge National Laboratory in order to understand the hazards of radiation." And the mammal of choice for this study was the mouse.

After describing some of the experiments involving exposing mice to radiation, and the effects it had on the mice, Davis recounts a **chance** discovery about a mouse colony that had not been exposed to radiation. These mice had a genetic mutation which resulted in vigorous autoimmune diseases. It took 6 more years to discover which area of the genome and which gene was involved. "There were twenty different genes in this region and the last of these to be tested individually turned out to be the single gene which had been altered to give these mice autoimmune disease. It was a gene named forkhead box P3, known as **Foxp3** (said Fox-P-3; the cumbersome name coming from a related gene first studied in fruit flies whose mutation results in the insect having a fork-headed appearance). A small fragment of DNA had been inserted into this gene by chance, preventing the gene from working properly and causing autoimmune disease."

This condition found in a mammal meant that it might play a role in humans. "Mutations in the human Foxp3 gene were identified in patients with a rare syndrome called IPEX (which stands for immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome). This syndrome – so rare that its prevalence is not known – is characterised by an overwhelming autoimmune attack on several organs."

This finding led to questions about the role of Foxp3. "In 2003, three research teams – Sakaguchi in Japan and two research teams in the US, led by Alexander Rudensky and Fred Ramsdell – discovered that the activity of the Foxp3 gene is not only linked to regulatory T cells, it is **essential** for their development and functioning. In fact, the activity of this one gene has the power to change a normal T cell into a regulatory T cell, transforming a cell's purpose from boosting to dampening an immune response. This in itself was a dramatic discovery: that a single gene, switched on or off, can change a cell's core nature. The reason that this one gene, Foxp3, is so powerful was found to be that it encodes for a protein that directly controls the activity of around 700 other genes. It is a hub in the network, **a master control gene**."

"Foxp3 was a far more reliable marker of these cells than anything used previously, and this allowed regulatory T cells to be tracked, isolated and systematically studied. The research that followed revealed that regulatory T cells safeguard against unwanted immune responses in more than one way. They secrete cytokines that dampen immune responses locally, and they can switch off the activity of another immune cell with a touch. One place in the body where it turned out that regulatory T cells are **especially abundant is the gut.** Here the immune system must be especially adept at knowing what's harmful and what's harmless, to distinguish 'salmon from salmonella'…"

Davis continues with an extended discussion about the gut microbiome and the immune system. "In addition, regulatory T cells in the gut have what is probably the hardest job in the immune system. Commonly, the immune system should react against bacteria found inside the body, but in the gut, regulatory cells have the task of **preventing any adverse reaction** to the bacteria that live there to our benefit, the gut microbiome. These bacteria help digest plant molecules that are otherwise indigestible, extract nutrients and synthesise vitamins, all in return

for a place to live. This is a symbiotic relationship that our immune system must preserve, not react against."

Davis describes the complexity of the gut microbiome, which contains trillions of bacteria and viruses, all potential pathogens, and which is exposed to additional bacteria and viruses through our ingestion of food and drink. "In order to adjust its behaviour appropriately and maintain different bacteria in the gut as necessary, the immune system switches on and off in response to small molecules called metabolites, which are by-products from the replication and growth of gut bacteria. Metabolites from desired bacteria dampen the sensitivity of immune cells, counteracting their tendency to switch on in the presence of bacteria. Likewise, if metabolite levels from favored bacteria fall, then the immune system takes this as a cue that unwanted, potentially harmful, bacteria have begun to displace the normal healthy flora. The immune system then kicks into action to defend us and our resident gut bacteria. In this way, our immune system does more than protect us from disease; it **directly maintains** the vital symbiosis between us and the bacteria that colonise us."

"The gut immune system also looks out for trouble more directly – by sensing molecules that in any normal situation would operate inside a cell and whose presence in the gut alerts the system to the fact that cells have been burst open, for example when bacteria or viruses leave a cell. Such molecules usually have nothing to do with the immune system when they are inside the cell – they might be important for cells to replicate or to move – but once outside they act as a bat-signal that there's a problem, and are referred to as **alarmins**."

This gives Davis the opportunity to present the "big idea" that Polly Matzinger, chief of the 'T Cell Tolerance and Memory Section' at NIH had. She "… had thought deeply about Janeway's suggestion, in 1989, that the immune system can't work solely by detecting things alien to the body but must specifically detect germs. She realised that even this needn't be the case: the body doesn't need to trigger an immune response in response to any virus or germ; it needs only to r**espond to germs that cause damage**. An effective immune system, Matzinger concluded, only needs to defend against things that are dangerous, and she proposed the overarching principle that the immune system works by **sensing damage to the body**."

Davis recounts that the publication of this idea in 1994 "caused a riot." Part of the issue was Matzinger's personality, as Davis amusingly recounts. But: "Today, Matzinger's idea is far less controversial: there is plenty of evidence that immune responses in the gut and elsewhere are **driven and shaped by damaged tissue**. My own view is this doesn't mean her idea should directly supersede other ideas about how the immune system works. **Rather, we must not expect everything the immune system does to fit any one overarching principle. The system discriminates between self and non-self, and it detects germs, and it responds to danger, and it does all these things concurrently – and messily. The immune system uses a collection of mechanisms which no single principle fully encapsulates**."

He gives an example in support of his admonition above: "… one type of alarmin that is released when the lining of the gut is **damaged** switches on **regulatory T cells** rather than normal T cells. This turns off the immune system rather than switching it on. Although damage indicates a problem that may very well be due to an infection that warrants an immune response, restraint is also needed to prevent the immune system from spiralling out of control and causing more damage. The level at which this alarmin dampens an immune response is altered by the levels of other molecules present, including cytokines that are themselves signifiers of levels of invading germs. **An inner universe** of small molecules, of metabolites, alarmins and cytokines, reflecting the presence of different gut bacteria, invading germs or damaged cells, all dial the activity of the immune system up and down."

Davis now introduces a surprising new element (but not surprising to me - I've covered this issue in several of my other study groups), namely diet. He notes that: "This complex blend of triggers and restraints is also tuned by the food we eat… **Diets high in fibre** have a wide range of overall effects on the body, from reducing blood pressure to lowering the risk of colon cancer. They also **affect our immune system** specifically; many of the molecules produced when bacteria break down soluble fibre stimulate the production of regulatory T cells. **At least in mice**, a high fibre diet increases the number of regulatory T cells, which helps protect against autoimmune disease."

Davis further explores the relationship between the gut microbiome and the immune system in humans. "It's possible that the average human microbiome has altered since the advent of modern hygiene, now that we are exposed to far fewer germs than our species would have been accustomed to in centuries past. It may be, for example, that it is less diversely populated than it once was, reducing the number of regulatory T cells we have. Having fewer regulatory T cells would lead to **less restraint on the immune system**, which could feasibly account for the **rise of all kinds of allergies**, including food allergies, as well as **autoimmune diseases**. This fits with the '**hygiene hypothesis**' first proposed by David Strachan, working at St George's Hospital in London. By studying a survey of over 17,000 children born in March 1958, he calculated, in 1989, that whether or not they had ended up with allergic hay fever correlated with the size of the family into which they had been born and especially with how many older siblings they had. He realised that, on average, **infections** would occur less frequently in smaller families. This led him to suggest that hay fever might be prevented by the contraction of infections early in childhood. In turn, this led him to suggest that, more broadly, allergies may become more commonplace with increased hygiene. His idea has guided our thinking about allergies ever since."

Davis cautions that this doesn't imply that we should be less hygienic, bathe less often, wash our hands less frequently, etc. There is no evidence that these practices increase the risk of autoimmune diseases. But "… there is evidence that children growing up on small farms are less likely to develop allergies. So something about a 'dirty' environment may help, and the important question is what, exactly?"

Studies have been conducted on Amish and Hutterite communities in the U.S. He notes the differences in their agricultural methods - Amish use traditional small farm, single family methods, and Hutterites use large scale, communal methods. The Amish live closer to their animals, and their children rarely have asthma. The Hutterites do not, and their children have a greater incidence of asthma among their children. "The fact that the Amish are less likely to get asthma corresponds with the hygiene hypothesis: stimulation of the immune system by microbes found on small farms might be what protects the Amish from asthma."

Davis goes on to describe other studies on the immune systems of both sets of children which seem to confirm the hygiene hypothesis.

He next considers another major factor of modern life that has an impact on the immune system "The use of **antibiotics** has also been linked with increasing the risk of allergies."

He notes that it's not just the over-prescribing of antibiotics for illnesses that aren't affected by them, like viral infections, but it's the presence of antibiotics that have seeped into our food and water, that are of concern, especially with the increasing prevalence of antibiotic resistant pathogens. "Far less discussed, however, is the possibility that antibiotics also damage our resident gut microbes and change a person's microbiome. The use of antibiotics by children, or mothers during pregnancy, has been linked with childhood asthma but this does not in itself prove that using antibiotics increases the risk of asthma; the correlation is very likely caused by

genetic or environmental factors which link families to both asthma and infections that require antibiotics. The consequences, if any, of antibiotics changing a person's microbiome remain unclear."

Exploring beyond the effect of antibiotics on our immune system, Davis notes the effect of geographical location on both the microbiome and autoimmune diseases. He recounts a study of children in Finland, Estonia, and Russia; childhood autoimmune disease is relatively common in Finland and Estonia, less likely in Russia. The study revealed particular types of bacteria common in Finnish and Estonian children, other types common in Russian children. The net effect? "…while a molecular component of bacteria dominant in the microbiome of Finnish and Estonian children is known to switch off our immune cells, the equivalent molecule in bacteria common in Russian children, which has slight differences, tends to have the opposite effect: it switches on immune responses. This fits with the idea that the make-up of a child's gut bacteria can impact how their immune system develops – and that bacteria common in Russian children may help protect against autoimmunity, because **switching on an immune response early in life** helps train the system to respond appropriately later in life." Davis notes that this finding has limited practical applications - we wouldn't want to deliberately expose children to germs. "But one acceptable intervention might be instead to control or supplement the food we eat."

This leads Davis to consider dietary solutions: "Vegetable fibre or supplements which encourage gut bacteria to multiply – so-called **prebiotics** – could feasibly nudge the state of our immune system to our benefit, but it's difficult when nurturing one kind of bacteria to ensure that a closely related but detrimental species of bacteria doesn't also thrive. Another idea is to ingest live bacteria, in yoghurt or other foods – so-called **probiotics** – which could also feasibly shift the make-up of our gut microbiome and in turn impact the state of our immune system." He notes our lack of evidence that these supplements actually work as advertised, but holds out hope that more sophisticated design of these supplements will improve their efficacy.

"One way that probiotics could become more sophisticated would be to use genetically modified live bacteria." These bacteria would be produced in a similar process that is used to produce bacteria that produce monoclonal antibodies - inserting specific genes into the bacteria which would then incorporate the gene into its own genome. For example: "In mice, bacteria engineered to produce a cytokine which normally comes from regulatory T cells can stop the symptoms of autoimmune disease. This has not yet been achieved in human clinical trials, but new medicines like this, and others we haven't yet conceived, will emerge as our understanding of regulatory T cells increases. And this is just the tip of an iceberg."

Davis begins to sum up: Before Sakaguchi's insight, "…the dogma was that immune cells capable of reacting against the body's own components were weeded out from the system, killed off in the thymus without ever reaching the bloodstream. But Sakaguchi and his contemporaries revealed the situation to be more complex than this. **The system specifically includes cells able to detect the body's own components**, which are there to safeguard against an immune reaction. We now know that this was just the tip of an iceberg because in fact, there are many types of T cell; far greater diversity than can be covered by the crude categories of 'normal' or 'regulatory' cells."

He goes on to rail against the classification scheme for immune cells, noting that it is too coarse, that the reality is much more complex, that types of immune cells are much more abundant. "Bluntly, it's hard to understand how the system achieves all that it does." He concedes that it may be impossible for any one person to understand the complexity of the

immune system, on a par with any one person understanding the complexity of the Google search algorithms.

Davis ends on a very positive, upbeat note: "The reason that we've begun to triumph – why it is not hyperbole to suggest that we are at the dawn of a health revolution – is that we have now identified some of the hubs in the system: cells and molecules that, when targeted with drugs that boost or halt their activity, dramatically shift the behaviour of the system as a whole. We saw this with anti-cytokines. Blocking only one cytokine, TNF, for example, can alleviate the inflammation that underlies arthritis by halting an entire cascade of effects – in this case by severing the feedback loop in which immune cells keep triggering one another into action, leading to an autoimmune attack. When drugs, foods, prebiotics or probiotics are developed to impact the behaviour or numbers of regulatory T cells, which are undoubtedly also a hub in the system, we will have new treatments for allergies and other autoimmune diseases."