OLLI 492: Human Immune System

Session 3: March 23rd **Summary and Observations**

Chapter 2: The Alarm Cell

The focus of this chapter is on the Canadian immunologist Ralph Steinman, and his discovery of and work on the dendritic cell. Like Janeway, he was interested in how an immune reaction got started; but his main concern was with the question "… how does the body decide to make an immune response with *the right level of caution*? This was a crucial question, because, he thought, if we knew how the immune system decided when and how it was appropriate to react, we would know how to regulate immunity and tackle the problems that occur when it goes awry, as in autoimmune diseases."

In 1970, Steinman "… joined Zanvil Cohn's lab at the Rockefeller University, New York, which already had a formidable reputation for studying immune cells." Initially he was focused on phagocytosis. But, "… in 1972, he turned his attention to another problem, which proved to be especially rewarding: the mystery of the *accessory cell*. At the time, the accessory cell was an idea rather than an actual cell, invented to account for an observation that was otherwise hard to explain: that when isolated immune cells (specifically T cells and B cells) were mixed with something known to be able to trigger an immune reaction, nothing happened. Presumably something else needed to be present for these immune cells to react, but nobody knew what was needed or why. The 'accessory cell' was the name used to refer to whatever that something else might be."

Using cells from the spleens of mice, "… Steinman **decided to look closely**…" at the hodgepodge of cells under a microscope. One type of cell caught his attention because they were "stellate and spiky-shaped." The author notes that this type of cell had been observed before "…in 1868 – by German biologist Paul Langerhans" who "…saw stellate cells in skin." "When Steinman watched the strange cells move, he saw that they could, in his own words, 'assume a variety of branching forms, and constantly extend and retract many fine cell processes'."

Davis next presents a diversion on 'perceptual blindness' - i.e., we only see what we're looking for, not the unexpected. He notes: "Steinman could have assumed that the strange-looking cells he encountered were variations of cells already known, or cells that had been affected in some peculiar way, perhaps by the process involved in isolating them." Instead, Steinman "looked closely;" the result was the serendipitous discovery of a new cell.

Steinman benefited from his position at Rockefeller University. "On the fifth floor of the building was, as Steinman himself wrote, 'probably the greatest concentration of cell biologists that have ever worked together in a contiguous space'…."

Among them was George Palade who "…developed the way in which scientists could look at cells with an electron microscope…" "Steinman used Palade's electron microscopes to peer inside his spiky cells." He decided to name them the "… dendritic cell – from the Greek word dendron meaning tree, on account of the cell's most obviously distinctive feature, the many branch-like protrusions emanating from its main body."

The author notes: "Though dendritic cells are found throughout the body – in blood, skin and nearly all of our internal organs – they are fairly uncommon in each place." Because of this, they would need to be isolated and concentrated to study them.

Here again, Steinman's position at Rockefeller University proved important. In another lab at Rockefeller, "… a team led by Christian de Duve were breaking up cells with detergents and other chemicals so that their innards could be separated and analysed. They did this using a centrifuge…. Using this method, de Duve's team had been able to identify a wondrous world of organelles – literally, little organs – inside cells."

"Steinman borrowed de Duve's methods and adapted the centrifuge to separate different types of cell instead of fragments of cells." Easier said than done. "It took years to work out how to do this…"

However, "The difficulty of the procedure and the fact that it required specific know-how – in the same way that you can't easily learn to ride a bike just by reading about it – probably helped Steinman in the long run: it meant he had dendritic cells all to himself, without much competition, for at least ten years." In addition, "Most scientists thought that Steinman had isolated a type of cell that had already been discovered – in 1882 – by Ukrainian zoologist Ilya (or Elie) Metchnikoff; a discovery which won him a Nobel Prize in 1908."

Davis gives a wonderful account of Metchnikoff's work and discoveries, essentially the existence of an immune system in animals. "He discovered, in other words, that some cells have the specific job of protecting an organism against disease: immune cells. On 23 August 1883, he publicly proclaimed that 'animals disarm bacteria by eating and digesting them'. Later, with the help of colleagues, Metchnikoff named the cells he had discovered as phagocytes, and their job of digesting harmful bodies phagocytosis, derived from the Greek for 'cell-eating process'. The type of cell best able to eat germs came to be called the macrophage, the 'big eater'."

To overcome this initial skepticism about the uniqueness of dendritic cells, Steinman engaged in an extensive set of presentations at scientific conferences. However, "One of Steinman's students recalls the reaction to him talking about dendritic cells at an international meeting as simply 'abusive'."

The author notes that the tide began to turn in the early 1980s. "Experiments which Steinman's team carried out in the early 1980s were crucial in persuading the community that dendritic cells were different. A student in Steinman's lab, Michel Nussenzweig, compared the reactivity of T cells when they were in the presence of other immune cells and found a unique potency of dendritic cells to switch on the reaction. In other words, Nussenzweig's work provided strong evidence that dendritic cells were the mysterious accessory cells."

With improvements in lab techniques and instruments, Steinman's lab was able to show that "…dendritic cells could stimulate an immune reaction at least a hundred times better than macrophages or any other type of cell." In addition, "In 1982, another student in Steinman's lab, Wesley van Voorhis, discovered human dendritic cells – all the early work was done with mouse cells – and showed that these too were potent at triggering immune reactions."

But Davis notes that acceptance of dendritic cells had not advanced Steinman's "… original question: how does the body decide to make an immune response with the right level of caution? Steinman had discovered that dendritic cells were potent in starting an immune reaction, but he didn't know why, how or what this meant for the working of the immune system as a whole. The path to really understanding the function of dendritic cells opened up only when Steinman and his team found out that the ability of dendritic cells to switch on an immune reaction could change."

An important role in this discovery was played by "…a dermatologist named Gerold Schuler" who joined the lab in 1984. "Crucially, Schuler found that when dendritic cells were freshly

isolated from the skin, they were indeed quite weak at triggering an immune reaction but when these same cells were cultured in the lab for two or three days, they became potent. This meant that dendritic cells do not exist in just one state; they exist in two states, 'on' and 'off'. The process by which they switch into the 'on' state Steinman called maturation, leading to the two states of dendritic cells being called mature and immature."

Although some dendritic cells may be "immature", they are not inactive. "They have at their surface many different **pattern-recognition receptors**…" for identifying pathogens. In addition, "… immature dendritic cells are good at phagocytosis, the eating process. A picture of the two states of dendritic cells therefore emerged in which immature dendritic cells efficiently sense and capture foreign substances in the body, while mature dendritic cells are powerful at switching on other immune cells to react."

Interest in dendritic cells blossomed in the late 1980s and early 1990s. More labs began work on these cells leading to a deeper understanding: "… dendritic cells were detected in organs such as the skin, lungs and gut, as well as in the spleen and lymph nodes, the small bean-shaped organs found in your neck, armpits, behind the knees and so on, which are filled with immune cells. (These are what you can feel swollen in your neck when you're ill from an infection; commonly called glands, even though technically they aren't.) The crucial discovery made from this line of research was that dendritic cells in tissues such as skin, lungs or the gut were found to be immature – while those in the spleen or lymph nodes were mature."

"From this, a narrative for what dendritic cells do in the body finally took shape. Immature dendritic cells patrol almost all of our organs and tissues but especially places exposed to the outside environment, such as our skin, stomach and lungs. These dendritic cells specialise in detecting germs, using the multitude of pattern-recognition receptors they carry. When an immature dendritic comes across a germ, it engulfs and destroys it. Having done so, it then switches into a different state: it matures. The mature dendritic cell makes a beeline to a nearby lymph node or the spleen, a depot jam-packed with other immune cells. There, in the lymph node, other immune cells are presented with fragments of the germs that the dendritic cells have engulfed. The right type of immune cells to deal with the problem then travel out from the lymph node to the site of trouble. All of this movement happens via the blood and the lymphatic system, a specialised system of thin tubular vessels which carry immune cells to lymph nodes, through fluid called lymph which is similar to blood but lacks red blood cells. Dendritic cells travel to a lymph node via lymphatic vessels, while T cells, for example, move out from a lymph node into the body's tissues via the blood."

The author next gives a compact description of the immune response to a cut or wound; I won't repeat that here.

Davis next describes the interaction of mature dendritic cells with other immune cells that initiates an immune response. "The stellate shape of the dendritic cell, with its multiple protrusions, has an explicit purpose here; it allows dendritic cells to connect simultaneously with multitudes of T cells." Most T cells will not have the complementary receptor, but: "When a T cell meets a dendritic cell that has engulfed a germ it is able to recognise, that T cell starts multiplying." The immune response is off and running.

"One T cell will divide to increase in number at least a hundred-or thousand-fold in the lymph node. (This expansion of cell numbers is why you can often feel lymph nodes swell in your neck when you have an infection.) Killer T cells – 'killer' being their formal scientific name, not just my attempt to spice up the story – move out of the lymph node to the site of the problem, to kill the diseased cells (such as those infected with a virus). Other T cells, meanwhile, called 'helper' T cells, stimulate other immune cells into action. We now know there to be different

kinds of helper T cell. Those formally called type 1 help fight bacteria, for example, while others, type 2, stimulate an attack against parasitic worms. Type 1 helper T cells mobilise macrophages, the big eaters, to deal with bacteria, for example. Type 2 cells, on the other hand, switch on a 'weep and sweep' response, in which (without being too graphic) gut cells weep mucus, and muscle contractions in the intestine sweep out live parasitic worms."

"In short, dendritic cells detect a problem and switch on the right kind of immune response to deal with the threat. In more formal language, they connect our innate immune response, the body's instant reaction to germs, to the adaptive immune response, which is longer-lasting and more precise, involving T cells and B cells. Other cells in the body, including macrophages, can also do this, but only when the body needs to reignite an immune response against germs that have been encountered before. Dendritic cells are crucial for firing up a precise immune response the first time a particular germ enters the body. They are our alarm cells."

Davis next presents an aside on the creative process for an artist or a scientist, noting the role of intuition, perseverance and plain luck. "Steinman discovered dendritic cells without any grand theory as to how they might trigger an immune response; he had no narrative that might have guided subsequent experiments."

Steinman's lab continued to delve more deeply into the workings of dendritic cells, to flesh out a more precise theory. This effort showed just how complex these cells are. "At first, all the experiments led Steinman, and others, to the view that dendritic cells were crucial in starting a precise immune response. But then, as different conditions and situations were tested, some experiments showed the complete opposite to be true; that the presence of dendritic cells could stop an immune response. Just as Steinman thought he had the game sussed, it turned out that he was only at level one and nobody knew how many more levels there were."

For example: "In one of the experiments that seemed to contradict the earlier research, dendritic cells were exposed to protein molecules alien to the body, but not whole germs. Treated this way, we would not expect dendritic cells to trigger an immune reaction: their pattern-recognition receptors would not detect germs and so the cells should stay immature. Indeed, these dendritic cells did not trigger a reaction in other immune cells, but something else did happen. Other immune cells that were exposed to these dendritic cells were rendered unable to participate in an immune reaction later, even when germs really were present. In other words, these dendritic cells triggered a state of apathy, or tolerance, in other immune cells, making them unresponsive."

Steinman didn't give up in the face of such complexity; he realized that "… understanding how the same cells initiate a reaction sometimes but stop it at others required us to understand the precise mechanism by which dendritic cells interact with other immune cells. Recall that dendritic cells engulf germs at the site of an infection and then, in the lymph node, show to T cells samples of molecules made by the germs. We now know that the way that they do this involves proteins encoded by a handful of especially important genes: the major histocompatibility complex (MHC) genes or, more simply, our compatibility genes. Proteins encoded by these particular genes protrude from the surface of the dendritic cell. They clasp small samples of other protein molecules from inside the dendritic cell, including molecules from any germs that have been engulfed, and put these up for show at the surface of the dendritic cell. T cells examine these samples of protein put up for display, looking for anything that has not been in the body before."

Davis continues in another aside: "… these proteins are special because the genes that encode them – and therefore the proteins themselves – vary from person to person. By and

large, we all have the same set of genes – the 23,000 genes which make up the human genome – but around 1% of the genome varies from person to person, such as the genes which affect our hair, eye or skin colour. Importantly, the genes which vary the most from person to person have nothing to do with our appearance but are part of our immune system. Variation in these genes gives the proteins protruding from our dendritic cells, presenting samples of what's currently inside those cells, a slightly different shape. This means that we each present a slightly different sampling of proteins made inside our dendritic cells. This is one reason why we each fare slightly differently when faced with any particular infection."

Returning to the original thread of the argument: "The detail here which helped us solve the mystery of the dendritic cell's ability both to trigger a reaction but also to prevent one is as follows: when a T cell locks onto something – something that has never been in your body before, presented within a groove of the compatibility gene protein – that alone is not enough to start an immune response. The T cell needs more evidence that an immune response is appropriate. Essentially, every T cell requires two signs that there is a problem. The first sign – Signal One being its formal name – comes from detecting a sample of a protein molecule that has never been in your body before. Signal Two comes from what are called co-stimulatory proteins. Co-stimulatory proteins are proteins held inside the dendritic cell shuttled out to the cell's surface when that dendritic cell's pattern-recognition receptors have locked onto a germ (and the dendritic cell changes from an immature to mature state). As a result, they are present at high levels only on the surface of dendritic cells that have come into contact with a germ, effectively providing a molecular mark that signifies that a particular dendritic cell has come into contact with a germ."

"In other words, the dendritic cell uses pattern-recognition receptors to detect the presence of a germ, or another sign of trouble such as fragments of an infected dead cell, and then the dendritic cell matures (or switches on) and presents samples of that germ to T cells. T cells which have the appropriately shaped receptor to lock onto something presented by a dendritic cell, i.e. something not from the body, require the presence of a co-stimulatory protein on that same dendritic cell as a signal to know it's from a germ, and that a response is needed. If a T cell locks onto something presented by a dendritic cell but doesn't see co-stimulatory proteins, it knows that it is reacting against something not from a germ. It may be a molecule that hasn't appeared in the body before for some other reason; maybe it is food or new proteins made during pregnancy or adolescence. In this situation, the T cell doesn't just abort an immune reaction; it switches into another state and becomes a tolerant T cell. This T cell is now unable to cause an immune reaction, even at a later moment. In this way, dendritic cells have the power to switch off T cells which could otherwise attack healthy cells or tissues."

Davis sums up: "… dendritic cells really do have a special place in the system. They have an ability to switch the immune system on and off – both to control our immunity against germs and to stop our immune system attacking healthy cells and tissues. Uncovering the workings of dendritic cells – an endeavour begun by Steinman, but later involving thousands of other scientists – eventually answered his original question as to how the body launches an immune response cautiously: it requires more than one signal before doing so."

Davis turns his attention to Steinman's belief that his research could lead to better treatments for disease. "Since dendritic cells are absolutely necessary to get an immune reaction started the first time a germ is detected in the body, they are effectively the body's natural adjuvant. We still don't precisely understand how chemicals such as aluminium salts work as an adjuvant, but it is likely they act on dendritic cells, making them switch from an immature to a

mature state as if a real germ were present. Surely, Steinman felt, we should therefore be able to use dendritic cells to create new kinds of vaccines against HIV, tuberculosis or cancer."

Furthering this goal, "Japanese scientist Kayo Inaba performed an experiment in Steinman's lab in 1990 which showed that a dendritic-cell-based vaccine could work…. she had found that dendritic cells could be switched on outside the body and then injected back into the body to ready the immune system. This was a new way to trigger an immune reaction and, potentially, a new kind of vaccine."

"The aim of a dendritic-cell-based vaccine, then, is to use these cells to switch on the body's defences against, say, a virus like HIV, the tuberculosis bacteria, or cancer cells. Inaba's experiments showed how this can work in mice. And as immunologists often quip, **that's good news for mice**." However, "Testing the procedure in humans is far more complex." Davis uses the example of fighting cancer to demonstrate the complexity.

Irony of ironies… In the midst of testing dendritic cells as vaccines and as a treatment for cancer, Steinman himself was found to have an advanced cancer; he was given just a few months to live. But this did not deter Steinman from continuing his work. Only this time, he would be using himself as a test subject. "In setting out to use dendritic cells to cure his own cancer, Steinman hoped that his life's work could save his life."

Davis describes the remarkable reaction of Steinman's friends and colleagues to the news that Steinman would be treating himself with his experimental technique. They all rallied around him. For example: "Steinman's first PhD student, Michel Nussenzweig, was by that time a professor at the Rockefeller University, New York. He took some of Steinman's tumour, removed during surgery, and grew it in mice for further analysis. Meanwhile, Ira Mellman, vice president of oncology research at the company Genentech, who had worked with Steinman as a postdoctoral researcher, had his team culture cells from Steinman's cancer, and then tried several drugs on it which he had access to but which had not yet been tested in clinical trials. In Toronto, another of Steinman's friends analysed the specific genetic mutations in his tumour. In Tübingen, Germany, another extracted protein molecules from the tumour to be used in experimental vaccines. One of the scientists who helped knew Steinman from having spent her high-school summers gaining work experience in his lab. Mellman recalls meeting with Steinman in his office to work out what they should try and not try: 'It was a totally natural scientific discussion, except we were talking about his tumour."

"In all, Steinman tried eight different experimental treatments, including three vaccines based on dendritic cells." Davis gives a good, condensed description of these efforts. Alas, to no avail: "… on 25 September 2011, having had dinner the night before with his wife, three children and three grandchildren, he was admitted to hospital for the last time." "In the end, he survived four and a half years, until 30 September, aged sixty-eight."

A further irony… Three days after his death, but before his death was widely known, the Nobel Committee announced that they had awarded Steinman a share of the Nobel Prize in Physiology or Medicine. When they were told of his death, they decided not to rescind the award. As Davis notes, "Steinman remains the only person ever to have received a Nobel Prize and not know it."

"By the end of his life, Steinman was widely celebrated by the large community of researchers studying dendritic cells. Like a tree known by its fruit, his name will forever be linked with the dendritic cell."

The author next offers several reasons for why dendritic cell vaccines are not more effective:

- "One reason why dendritic-cell vaccines are not more effective is that tumours have evolved ways to thwart the immune system."
- "A second problem is that when dendritic cells have been switched on outside the body, arming them to trigger an immune response when reintroduced, they tend to lose their ability to migrate within the body."
- "A third problem with dendritic-cell vaccines is that, as recent discoveries have shown, there are in fact, many types of dendritic cells."

Davis notes: "… this makes the immune system akin to an ecosystem; cells in different habitats have many similarities but also vary and may adapt if they relocate." This is a current frontier of research, and may yet yield positive results. "There may be a subtype of dendritic cell that is especially potent at triggering immune responses in the context of a vaccine."

Davis sums up: "Within his lifetime, Steinman's gift to humankind wasn't new medicines; it was a new consciousness of the human body. For centuries, we have known that blood circulates in the body, distributing oxygen and nutrients. Steinman, and the thousands of scientists around the world who eventually worked on dendritic cells with him, unravelled the details of another great dynamism within the human body: that different types of immune cells shuttle between our organs and tissues, into lymph nodes and out again, to defend us continuously and vitally."