OLLI 492: Human Immune System

Session 4: March 30th **Summary and Observations**

Chapter 3: Restraint and Control

In this chapter, Davis reaches back into ancient history, the **summer of 1956**, to begin his tale of the discovery of the biochemicals that control and influence the immune system. As with the other discoveries with which he has regaled us, the story begins with the serendipitous pairing of two unlikely collaborators, and a mystery. The odd couple are the Swiss scientist Jean Lindenmann who came to work in the lab of the British scientist Alick Isaacs that summer.

And the mystery? "Before meeting Lindenmann, Isaacs had, for many years, been trying to solve a long-standing mystery about viruses… why the presence of one virus seemed to block the growth of another… [the mystery] wasn't studied systematically until 1937, when it was established that monkeys infected with one type of virus, Rift Valley fever virus, were protected against infection with another virus, yellow fever virus. Even for cells growing in a culture dish, when two different viruses were added, often only one grew well."

Davis notes that "The hot topic at the time, especially in the Mill Hill institute [where Isaacs worked], was how flu spreads in an epidemic." Isaacs team made significant contributions to this issue, but he nevertheless also pursued a solution to his mystery.

Lindenmann and Isaacs developed two hypotheses to explain this phenomenon.

"One possibility was that a protein molecule that viruses were known to depend on in order to gain entry to cells got used up, or removed, when one virus entered a cell, preventing a second virus gaining access to the same cells. Another possibility was that a molecule required by a virus in order to replicate might get used up, meaning that a second virus could enter the same cells but would be unable to multiply. They realised that either of these answers would be big discoveries if proved true, because as well as revealing how viruses work, they would expose a way in which viruses are vulnerable."

I won't go into the details of the experiment they devised to test their hypotheses. It involved infecting membrane cells of a fertilized chicken egg with the flu virus, and mixing the virus with red blood cells. Once the virus transferred its genetic material into the cell, the virus outer coats would remain on the red blood cells, and could therefore be harvested. "The red blood cells with virus coats stuck to them could then be tested to see if they could still stop a viral infection when added to fresh chicken cell membrane. If so, they reasoned, it would demonstrate that the outside coat of a virus is what blocks a second infection, as opposed to the genetic material of the virus."

"They found that red blood cells that had been coated with virus and washed off from chicken membrane cells could indeed still stop another virus infection. This seemed to fit with the idea that the outside coat of a virus was the important factor for blocking a second infection"

However, upon further analysis, it appeared that viruses may have detached from the red blood cells during the experiment. "It seemed possible that f**ully intact virus** might have detached from the red blood cells, which was what was blocking a second infection. If so, their experiment hadn't revealed anything new at all. By tackling this worry with a **new experiment**, they struck gold; actually, something far more valuable than gold."

"They decided to repeat the experiments without the complication of adding red blood cells. Now they found that the **liquid** taken from a test tube which contained virus and membrane

cells was also able to stop virus from infecting fresh cells. **Something in the liquid** – just the liquid – stopped viral infections."

"Isaacs suggested that something able to **interfere** with viruses might have been generated in the liquid… Lindenmann chose to name whatever it was that was causing the interfering activity ***interferon***… On 6 November 1956, just over two months since they started working together, Isaacs titled a new section of his lab notebook: 'In search of an interferon'. And the hard work began."

The pair were in uncharted territory. "Like detectives arriving at the scene of a crime, not quite sure what they were looking for, they probed the liquid's capabilities for any sort of clue…. Over time, they ruled out uninteresting and circumstantial explanations and began to grow confident that something as yet unidentified, equipped with the power to stop viral infections, was actively at work; in other words, that there really was an interferon."

"By the end of February 1957, they decided that they had accumulated enough evidence to warrant writing up their claim of a new cell-derived, virus-induced factor which could interfere with virus replication."

After publication, they began to speak at conferences about their find; they were met with a great deal of skepticism, with some justification. "The early experiments were complicated – cells and viruses were incubated together, liquid siphoned off and reused – and it was open to debate as to what exactly in this process produced the interfering factor. Also, the complexity of the experiments meant it was hard for other scientists to reproduce the results."

Davis notes that "… despite the objective tone of scientific papers, the pursuit of new knowledge is an intensely personal endeavour."

Davis next describes how badly Lindenmann and Isaacs were treated. Their professional lives and careers were never the same afterward. In addition, "There was great pressure on Isaacs, from the government, the scientific community and the public, to prove that interferon was real, that it could work as a drug and to obtain a patent for it. He suffered deeply from the stress and, unknown to his colleagues, attempted suicide at least twice."

Isaacs assigned Derek Burke, a young chemist in his lab, the task of purifying interferon from the liquid. "Isaacs thought this would take Burke about six months and then his ideas would be proved right. But purifying interferon turned out to be a Herculean task. The liquid siphoned off from cells and virus contained minuscule amounts of interferon and Burke filled up twelve notebooks ploughing through chemical processes in his attempts to isolate it. In hindsight, it was hopelessly naïve to think this would take six months. **It took fifteen years**."

Davis next describes how Isaacs, beginning in early 1964, suffered a series of brain hemorrhages that ultimately led to his death in January 1967 at the age of forty-five.

Near the end of his life, "… a series of small clinical trials of interferon were disappointing and pharmaceutical companies lost interest. Soon after he died, however, the promise of interferon was revived by **cancer** research. Most cancers have nothing to do with a viral infection but there are a small number of viruses that have been associated with cancer."

Cancer researcher, Ion Gresser, working in Paris, showed that interferon (or at least, the "liquid" from a viral infection - interferon had not yet been isolated and purified) could stop cancers caused by a virus. Surprisingly, he also "… performed the same test on other types of cancer that have nothing to do with a virus, thinking that these more common types of cancer would

not be affected. Unexpectedly, he found that animals injected with all different types of cancer cells survived when treated with interferon. In 1969, he reported that, at least in mice, interferon could cure cancer."

Apart from his work on cancer, Gresser suggested another way of looking at interferon. "In one of his lower-profile research papers, published in December 1961, Gresser noted that, like other cells, human white blood cells mixed with viruses also led to the production of interferon. He speculated that this might play some role in the body's immune defence against viruses and suggested that the production of interferon might be used as a diagnostic test for the presence of a viral infection."

This observation proved inspirational for one researcher: "Finnish scientist, Kari (pronounced 'Kory') Cantell… reasoned that although most human cells mixed with viruses would lead to the production of interferon, perhaps human white blood cells are especially good at making interferon, and if so, these cells could be used to produce interferon in large amounts in the lab. "

While there was an element of serendipity in Cantell's experiments, what really stood out was his persistence. "Cantell tested his idea on a virus that he happened to have in his freezer called Sendai virus, which is a little like the flu virus, named for the Japanese city where it was discovered. We now know that Sendai virus is especially effective at getting white blood cells to make interferon. Had he used another virus, or even a different strain of the same virus, his first experiment would have failed and he might never have persevered. As it happens, in his first experiment – begun on 8 May 1963 – white blood cells produced ten times more interferon than any other type of human cell he tested." After a good start, perfecting the process for extracting and purifying interferon nevertheless took him "**nine years**."

It was a complex process: "Cantell found that he could extract interferon by stirring an initial crude preparation in cold acidic alcohol and then slowly raising the pH of the liquid by adding other chemicals. Impurities came out of the solution quicker than interferon, and could be removed by centrifugation. The whole process had to be repeated several times." It was a very long, complex and unusual process that proved effective in purifying interferon. "It had been fifteen years since Isaacs and Lindenmann reported interferon and, just when public interest in the topic was at a low point, Cantell found a way to purify it, opening the way to put their thesis to the test once and for all."

Cantell now had a monopoly on producing interferon, while other labs struggled to duplicate his process. Davis describes the lengths to which researchers went to obtain quantities of interferon for human clinical trials of its effects on cancer. But given the very limited supplies of interferon, the trials were not rigorously controlled and only included a handful of subjects. The early results were positive but mixed. But the political climate in the U.S. at the time skewed the perceptions of the trials: "The US cancer research community were also thrilled by these early results with interferon because they were under pressure to deliver new medicines after President Nixon signed the 1971 'war against cancer' Act."

"The situation began to change in March 1978 when Cantell took a call from Charles Weissmann, from the University of Zurich, whom he didn't know. The revolution of genetic engineering was in the air, the biotech industry was expanding. San Francisco-based company Genentech had just shown that a human gene could be inserted into bacteria, and these genetically modified bacteria would then produce the human protein encoded by that gene. This works because the chemical machinery which makes proteins inside cells is essentially the same in bacteria as it is in us: bacteria treat an inserted human gene just as they would any other gene and produce the protein that the gene codes for."

In 1982, Genentech receives FDA approval for the first genetically engineered medicine, human insulin.

Davis goes on to describe the process that I've referred to as the "Central Dogma", namely that DNA is transcribed into messenger RNA (or mRNA), and mRNA is translated into proteins. If the gene that codes for interferon could be identified, then it could be inserted into bacteria which would then produce large amounts of interferon. The process involved capturing the mRNA for interferon and reverse engineering the DNA gene that produced it. This process was at the frontier of biotechnology.

Cantell collaborated with Weissmann, but was unaware of the fact that "Weissmann led the work as an academic-entrepreneur and co-founder of the biotech company Biogen…. Biogen announced at a press conference on 16 January 1980 that they had produced interferon from genetically modified bacteria."

By 1982, clinical trials with interferon's effect on tumor size proved disappointing. Davis observes: "Many drugs look hopeful in a handful of patients only to fail when tested more carefully on larger numbers of people."

The increased availability of interferon led to increased trials and experiments. Our knowledge about interferon increased dramatically. But: "By 1984, the consensus was that interferon was not going to be a cure for cancer in any simple way…. it was also clear that there wasn't just one type of interferon…. And several different teams discovered that interferon wasn't the only type of protein molecule able to influence immune cells."

"… the existence of interferon opened the world's eyes to a whole host of soluble proteins like it which are in the body for the same purpose: communication between cells and tissues and coordination of the immune system. We now know that there are over a hundred different proteins like interferon, some of which have been studied across thousands of labs while others have been discovered only recently. Collectively they are called **cytokines**; they are the immune system's hormones. Our immune cells bathe in a cacophony of cytokines – some switch the system on, others turn it off, many nudge its activity up or down a shade. Their purpose is to shape an immune response to fit the type of problem, say a viral or a bacterial infection, and connect the immune system to other body systems. Their actions are incredibly complex – there are cytokines that regulate the cytokines – but as we shall now see it is hard to overstate their importance in how the body works or their potential for new medicines."

Davis observes that: "All human cells can be invaded by microbes and this is often damaging… To defend against this, **almost all human cells** can sense when they have been invaded by a germ, using **pattern-recognition receptors** to detect their telltale signs. As we have seen, some types of pattern-recognition receptors detect a germ by locking onto a molecular shape which is alien to the human body, such as the outer coating of a virus or bacteria. Other pattern-recognition receptors detect the presence of a germ because they lock onto molecules, such as DNA, which are not alien to the body but are in a location where they shouldn't be, giving away that they are part of an invading germ. Dendritic cells have a vast array of different pattern-recognition receptors, which makes them especially adept at detecting different kinds of invading germs, but almost all cells in the body have some types of pattern-recognition receptor. When any cell's pattern-recognition receptor locks onto the telltale sign of a germ, **this triggers the cell to start producing interferon**. In this way, almost

any type of human cell can be induced to produce interferon when, for example, it is infected with a virus."

"Interferon turns the infected cell, and other cells nearby, into a **defensive mode**. It does this by switching on a set of genes appropriately called the interferon-stimulated genes. These genes produce proteins which help stop bacteria and other germs, and are especially potent at dealing with viruses: they can block viruses from being able to enter nearby cells, stop viruses already inside cells from getting into the nucleus of cells (where they need to go to replicate), and prevent viruses from usurping the cell's machinery to make the proteins needed for new copies of the virus."

"In the case of some viruses, this response – our innate immune response – is enough to keep the infection under control, but often this only dampens an infection for a few days until our adaptive immune response – led by our T cells and B cells – develops to eliminate the problem completely and provide long-lasting immunity. One reason that an interferon-stimulated response often can't wipe out an infection is that viruses, and other types of germs, counteract its effects…. our body is locked in an **everlasting arms race with minuscule germs**."

"We each respond to germs in the same way, but only to a first approximation. One reason that some of us are more likely to suffer especially badly from a flu infection is because of a variation in our interferon response genes." Davis cites the example of people with a non-functioning IFITM3 gene "Normally, the protein made from the IFITM3 gene interferes with how the influenza virus enters cells, though precisely how is not yet understood." There are severe consequences for those with a non-functioning IFITM3 gene: "In 2012, the non-functional form of this gene was found to be especially common in people hospitalized by an influenza infection. Those in intensive care were seventeen times more likely to have the defective gene."

However, "… most people with a dysfunctional IFITM3 gene will still be able to fight off a flu infection without a problem, as it is one of many components of our immune response. In fact, it may even be beneficial to lack a functional IFITM3 gene in other illnesses, such as those conditions in which an immune response is the cause of the problem."

Davis goes on to note that, although we don't fully understand the implications of a non-functioning IFITM3 gene, we may be able to exploit what we do know. He cites the potential for prioritizing flu vaccine administration based on genetic make-up, i.e., prioritizing those most at risk. And, we may be able to use this knowledge to boost the interferon response in the absence of a vaccine.

Davis summarizes the results for interferon: "Although interferon never lived up to its early hype as a cure for cancer, it is important in the treatment of melanoma and some types of leukemia, usually given as an injection several times a week…. The chief reason that interferon doesn't work as well as we once hoped is that it doesn't stop cancer cells directly. We know now that most, if not all, of the way interferon helps fight cancer is by stimulating our immune system."

He places interferon in the context of the complex family of proteins of which it is a member: "There are many different types of interferon – at least seventeen – produced by different cells in the body. Most of our cells can produce the type of interferon that Lindenmann and Isaacs discovered – nowadays referred to as interferon alpha – to limit the spread of an infection. Today, interferon alpha forms part of the treatment for hepatitis B and C infections. Other forms of interferon are more specialised: interferon gamma, for example, is mainly produced by some types of white blood cell in order to amplify an ongoing immune response. The genes switched on by each type of interferon are being catalogued in an ever-expanding online database. Many of the other cytokines, discovered after interferon, are called **interleukins**, so named for

being the proteins that act between (inter-) leukocytes, a formal name for white blood cells. Abbreviated to IL, each type of interleukin is assigned a number, IL-1, IL-2, IL-3 and so on, currently up to IL-37."

"Each has a multitude of specific effects and here's just one example: IL-1 acts on, among other cells, neutrophils, which are the most abundant immune cells in the bloodstream. Neutrophils are recruited to a cut or wound within minutes. They can engulf germs and destroy them directly. But one of the especially wondrous things that neutrophils do for our defence is that they shoot out a sticky web, or net, made from strands of DNA and proteins, to capture germs moving by.… These webs contain antimicrobials which kill the captured germs. Neutrophils have a short lifespan, just a day or so in the blood, but at the site of an infection, the cytokine IL-1 increases their lifespan dramatically so that they can battle on, shooting out webs and killing germs for up to five days."

"To take a second example, IL-2 has a dramatic effect on other white blood cells, such as Natural Killer cells, a type of white blood cell that is especially adept at killing cancerous cells and some types of virus-infected cells…. When IL-2 is added, these cells elongate from a sphere into a Y-shape and change from being inactive in the culture dish to literally crawling about, the front end of the cell pushing against the surface of the dish while the rear part lets go, propelling the cell forward, probing for diseased cells to attack. If a Natural Killer cell meets a diseased cell, a cancer cell or a virus-infected cell, for example, it will latch onto it, flatten up against it and, within a few minutes, will kill it. The white blood cell then detaches itself from the debris of the dead diseased cell – which looks like a bubbling mess down the microscope – and searches for others to attack."

But some cytokines have quite the opposite effect on the immune system: "One of the cytokines which **turn off** immune responses is IL-10. Discovered in 1989, isolated in 1990 and studied by thousands of scientists since, we now know that this cytokine helps protect the body against unwanted immune reactions. IL-10 curbs inflammation when an infection has been eliminated and signals for the body's healing process, the repair of damaged tissues, to begin. IL-10 is also important in our gut, where it keeps immune cells in a relatively inert state to prevent unwanted reactions against harmless bacteria. Mice genetically altered to lack IL-10 suffer from an inflammatory bowel disease. In humans, an overreactive gut immune system can cause Crohn's disease and ulcerative colitis.…"

"Our knowledge of cytokines leads to a big idea for medicine: to manipulate their levels in the body in order to boost the immune system to fight infections or cancer, or dampen immune reactivity as treatment for an autoimmune disease."

Picking up on this potential, Davis next presents a lengthy description of the work of cancer researcher Steven Rosenberg of the NIH: "One pioneer – some say *the* pioneer – in boosting the body's immune response to cancer is Steven Rosenberg…. Rosenberg became the chief of surgery at the National Cancer Institute, Bethesda, USA, on 1 July 1974, at age thirty-three, overseeing nearly a hundred staff and an annual budget of millions of dollars. He has stayed there ever since – because he feels it is 'the ideal place in which to do solid basic science and take it to the bedside"

Early in his career, Rosenberg became convinced that the immune system was critical to curing cancer. One approach he tried was to culture a patient's immune cells outside the body, and then re-infusing them back. "Rosenberg built upon the discovery that the cytokine IL-2 could be used to stimulate human immune cells to multiply."

After a string of failures with this approach, Rosenberg succeeded in curing a patient, Linda Taylor, of metastatic melanoma (his 67th attempt). However: "Trials with larger numbers of patients showed that IL-2 was the important ingredient in Rosenberg's treatment, not the immune cells. But alas, it soon became clear that IL-2 is not a wonder drug. Less than a year after Rosenberg's success with Taylor, another patient given high doses of IL-2 died." Although the patient had many tumors and would have died in any event, it was Rosenberg's treatment that was the cause of his death.

"IL-2 seemed to offer patients either spectacular success or tragedy – and neither Rosenberg nor anybody else could predict which it would be. Various clinical trials, large and small, have since proven that IL-2 is best in treating people with melanoma or advanced kidney cancer"

Davis offers some observations on the situation: "Why IL-2 works for only some types of cancer is not clear. Melanoma, the type of cancer which Taylor had, involves more mutations than most other cancers. So one possible reason why IL-2 helps against melanoma more than most other cancers is that their large number of mutations mark out melanoma cells as being especially different from healthy cells, making them relatively easy for the immune system to detect and react against. Why some patients respond well to treatment with IL-2, but others don't, remains, unfortunately, unknown. It is possible that the treatment works best in people with a level of immune reaction already ongoing against their tumour, there to be boosted by the treatment."

Davis sums up: "Altogether, this band of pioneers, from Lindenmann and Isaacs to Gutterman and Rosenberg, discovered the existence, and then the power, of cytokines. They seeded an enormous scientific endeavour – cancer immunotherapy – which now has hundreds of branches, each studying a different way of boosting our immune response to cancer. A multitude of cancer treatments, with many more on the horizon, are the outcome."