RALPH STEINMAN AND THE DENDRITIC CELLS

By Michael Simm

The Nobel Prize in medicine has been awarded annually for the past 110 years, but probably never before has the announcement of the winning scientist cast that individual's work in such a dramatic light.

According to the official statement made by the Karolinska Institute in Stockholm, Canadian immunologist Ralph M. Steinman was inducted into the pantheon of the scientific elite for discovering dendritic cells with Zanvil A. Cohn, who died in 1993, and for clarifying the role of these cells in adaptive immunity. The co-winners of the prize in 2011 were Bruce A. Beutler (US) and Jules A. Hoffmann (France).

Directly after the announcement, the jurors and the general public learned that Steinman had died of pancreatic cancer a few days earlier, having fought the disease until the end with the very cells he had discovered. This was 'the saddest Nobel Prize of all time' wrote the *Frankfurter Allgemeine Zeitung* on 3 October 2011. Yet Steinman's tragic death might well have marked a turning point in the treatment of cancer: the researcher lived for four and a half years with an illness that usually causes death within a few months. Caution is advised, as Steinman himself would hardly have regarded a case history like this as proof of the method's effectiveness, but there is much evidence that, almost 40 years after the discovery of these 'generals' of the immune system, customized tumour vaccines based on dendritic cells could become a viable option for treatment.

In 1970 Steinman went to New York's Rockefeller University as a postdoctoral fellow, initially working in the lab of his mentor, Zanvil A. Cohn. He remained at this institution until his death, becoming director of the Center for Immunology and Immune Diseases in 1998. Shortly after he arrived, he discovered unusual cells in the spleens of lab mice that he called 'dendritic' because of their branch-like structure. Although German pathologist Karl Albert Ludwig Aschoff had described these cells as components of the reticuloendothelial system as early as 1924, no one had attached any importance to them before Steinman.

At the time, Steinman tried unsuccessfully to convince his colleagues of their significance. For years most scientists did not even believe they existed, recalls Professor Gerold Schuler, who worked as a postdoctoral fellow in Steinman's lab between 1983 and 1985. Over the last few years, Schuler, who directs the Dermatology Clinic at Erlangen University Clinic, has treated more than 400 melanoma patients using an experimental vaccine made of modified dendritic cells. The long-term results of these trials have not yet been published, but one finding is already known: one in four patients was still alive after five years. This means that the life expectancy following administration of this type of cancer vaccine is considerably longer than the average 10-month survival time following the standard treatment in an advanced stage of the disease. Schuler is in the best of company with →

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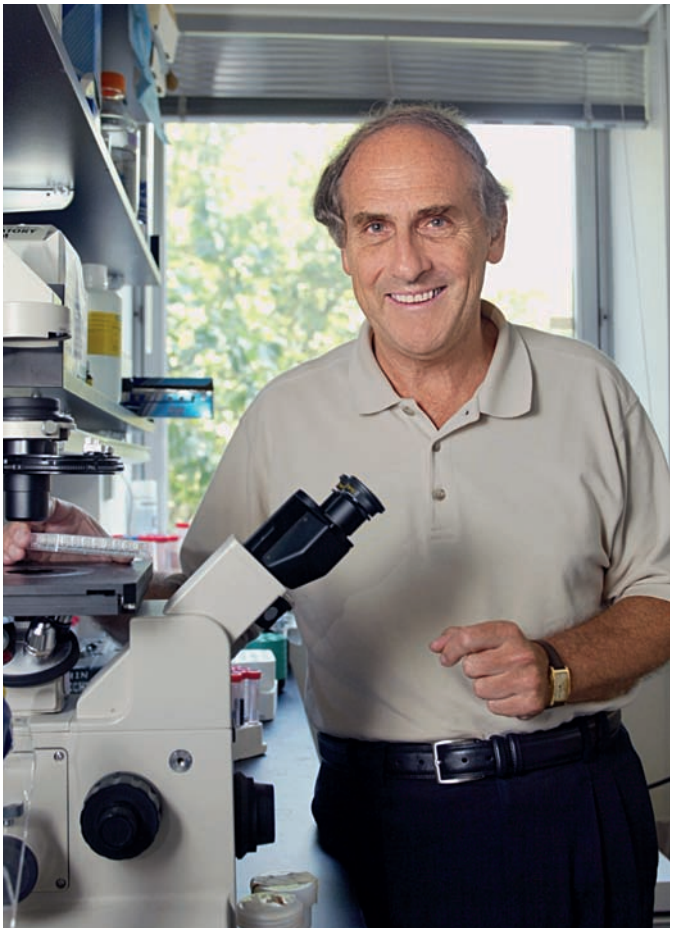

Immunologist and Nobel Prize winner Ralph M. Steinman

these experiments: around 400 studies of similar treatments have been registered at the US clinicaltrials.gov database. And a look at the PubMed literature database reveals that dendritic cells have long since entered the scientific mainstream. The MeSH term yields more than 30,000 articles – although its indexation only dates back to 1986.

For decades the prevailing school of thought among immunologists was that the interaction between two types of white blood cells – macrophages and B cells – played a primary role in activating the immune system. But Steinman and Cohn were able to demonstrate that the immune system's most powerful weapons – the T cells – were at least 100 times more effective when they came into contact with dendritic cells, which present antigens – the protein components that stimulate the formation of antibodies – on their spiky arms.

Today we know that immature dendritic cells take up and process antigens, which, after a maturation process lasting several days, are displayed on their surfaces together with other stimulating molecules. In contrast to the rather stationary macrophages, dendritic cells are highly mobile within the body. They migrate to the lymph nodes, where they interact with circulating T cells. The antigens the dendritic cells display 'teach' the T cells how to recognize and attack enemies within the body. It is the denditric cells, then, which seem to be critical in unmasking the foreign antigens that T cells alone are incapable of detecting and attacking.

But Steinman's discovery of the significance of dendritic cells would have been a mere footnote in the history of immunology had not other researchers contributed decisively to clarifying their ontology (development). Their work laid the foundation for cultivating these 'commanders' of the immune system, producing specific vaccines against cancer and thus ensuring the 'greatest possible benefit for humanity,' which Alfred Nobel defined in his will as a criterion for the annual presentation of his award.

As early as 1967, Hinrich Peters and Dieter F. Hülser of the Max Planck Institute in Tübingen addressed the question of how physical contact between different cell types in the immune system led to the stimulation of lymphocytes. The researchers also set out to find a 'third cell type'. Inspired by Steinman's insights into dendritic cells, Hinrich Peters, who became a professor in Göttingen in 1982, soon began concentrating on identifying their progenitors.

While Steinman and other researchers were still trying to enrich the coveted immuno-commanders in various tedious purification procedures, Peters and his colleagues became the first to describe the origin of dendritic cells. In a 1987 publication that was received with great scepticism, the scientists wrote that the 'veiled accessory cells' stem from blood monocytes.

'At the time, the antigen-presenting cells that are a focus of immunology today were relegated to the appendices of the discipline,' says Robert Gieseler, a former PhD student of Peters. One reviewer, he recalls, commented on a rejected manuscript with the words: 'Someone apparently examined a few starving macrophages here.' That these 'starving macrophages' could be generated by adding the GM-CSF growth factor and Interleukin 4 to blood monocytes was a discovery that Peters' team and BIF fellowship holder Jörg Ruppert described in publications between 1991 and 1993. With this knowledge, dendritic cells could now be generated rather easily in large quantities, which in turn allowed for numerous experiments that propelled the field. After a long delay, the discoveries of Peters' group ultimately swayed Steinman, who had originally postulated that dendritic cells originated from a developmental line that was independent of blood monocytes and lymphocytes.

Although the 'family' background of dendritic cells has not yet been fully clarified, no one doubts today that most descend from blood monocytes and that this line provides the most effective agents for antigen-specific adoptive immunity. As the quickly

Photo: Rockefeller University

growing community of researchers characterized how the maturation and differentiation of dendritic cells was precisely regulated by a large number of signalling molecules, more and more options emerged for using them as tumour vaccines.

In April 2010, Sipuleucel-T became the first vaccine involving dendritic cells to receive official approval from the US Food and Drug Administration (FDA). For this immunotherapy against advanced prostate cancer, mononuclear cells must first be isolated from the blood of individual patients. The coveted antigen-presenting cells are enriched through centrifugation, incubated for 40 hours in the lab together with a recombinant fusion protein (PA2024), and injected into the patient's bloodstream at 2-week intervals. PA2024 is a bipartite construct in which the GM-CSF growth factor, as an activator, is coupled to prostatic acid phosphatase (PAP) through genetic engineering. The goal is to teach the T cells to recognize and attack the PAP peptide as a tumour antigen on prostate cancer cells.

A full treatment cycle with Sipuleucel-T consists of three injections. In the registration trial leading to approval, it extended the lives of the 341 recipients by an average of 4.1 months compared to the results for the 171 patients in the control group. At 31,000 dollars per injection, it is one of the most expensive cancer treatments on the market and has provoked heated debate in the United States as to the affordability of such medications.

A meta-analysis of 29 clinical studies on prostate and kidney tumours by the Cologne University Hospital suggests that the principle seems to work. In cases of malignant brain tumour (glioblastom), vaccines based on dendritic cells have also become one of the most frequently tested immunotherapies, according to findings by an additional overview of studies from 2010, published in the journal *Clinical and Developmental Immunology*. Although the results seem encouraging, hardly any of the experts have dared to speak of a cure. Apparently the tumour antigens that are unmasked by the vaccination become invisible again to the body. Cancer cells that modify their surface structure through mutations escape the activated T cells or form substances that are interpreted as stop signals by the lymphocytes or that slow down the dendritic cells. Furthermore, rapidly growing tumours form a sort of protective cover that shields the cancer cells from attackers.

Steinman not only foresaw all these problems but described them in a survey article for *Nature* co-authored with his friend, colleague and rival Jacques Bancherau from the Baylor Institute for Immunology Research in Dallas, Texas. This piece was written at about the same time that Steinman was diagnosed with advanced pancreatic carcinoma. The two scientists noted that, like infections, cancer could evade dendritic cells and even utilise them for its own purposes. They made dozens of proposals for fine-tuning these cells, including 3 different vaccines that were derived from Steinman's tumour and were tested on his own body. Together with 5 additional experimental therapies, all of which were submitted to and approved by the FDA, this process was probably the 'ultimate experience in personalized medicine', says Steinman's colleague Jedd Wolchok, an oncologist at the neighbouring Sloan-Kettering Cancer Center in New York. Although dendritic cells played a leading role throughout this drama, it was the visionary immunologist Ralph Steinman who served as its director, because, as he himself wrote in the survey piece, 'The patient sets the standard for the quality of knowledge that is required to understand many aspects of the disease and its treatment.'

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Our genes are like books in a library: to answer the question 'What is life', it takes the right reader to read the right book at the right time.