Ralph Steinman: Dendritic cells bring home the Lasker

Ralph Steinman is perhaps best known as a codiscoverer of dendritic cells (DCs) and as a founding father of the research area that these cells have spawned. For his discovery, Steinman was recently awarded the 2007 Albert Lasker Award for Basic Medical Research. Yet the man behind the research holds his praise for the many other scientists—in the U.S. and abroad—who have further advanced the therapeutic promise of DCs.

A successful battle against a foreign antigen is the outcome of a precise series of events—the outsider must be recognized, captured, and delivered to just the right group of immune cells. At the same time, the immune system must be prevented from reacting to its own antigens. More than three decades after the discovery of DCs, it is now clear that these cells are equipped to handle both tasks. DCs can sample antigens anywhere in the body, deliver the information to lymphoid organs, and activate different types of lymphocytes depending on the job at hand—defense versus tolerance. DCs are now being used to enhance resistance to pathogens and cancers and to treat allergies and autoimmune disorders. Ralph Steinman may be credited with the discovery of this unique cell type, but he is quick to share the accolades with his colleagues.

Career change

Steinman was a physician at Massachusetts General Hospital (Boston, MA) in 1968 when his interest in immunology was first piqued by a lecture series on "new cellular immunology." Macfarlane Burnet's clonal selection theory, which proposed that every antigen is recognized by a single, clonal immune cell, was then about a decade old and had become the bedrock of immunology. B and T cells had been identified as the perpetuators of antibody- and cell-mediated immunity, respectively, and their mechanisms were being rapidly uncovered. These discoveries and their potential impact on clinical practices appealed to Steinman. But despite all the advances in understanding the immune system since Burnet's time, Steinman notes, one fundamental question remained unanswered: "We still didn't know how an immune response got started."

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> –Ralph Steinman

As a physician, Steinman had firsthand experience with this mystery. The immune systems of tuberculosis patients and of healthy humans who had been vaccinated with the tuberculosis vaccine "recognized" the bacterial antigens in skin tests. Those who had never been exposed to the bug did not. "We had the beautiful clonal selection theory, which proposed that a preexisting clone would recognize and respond to any antigen that comes along," says Steinman. "But there was this big glaring gap where, in some instances, a foreign antigen was simply not inducing a response [in nonexposed individuals]."

To understand how antigens prompt immune reactions, Steinman began postdoctoral work in the laboratory of Zanvil Cohn at Rockefeller University. Cohn was a pioneer in the study of macrophages and their role in taking in and breaking down proteins and infectious agents. In this lab, Steinman began by studying how macrophages captured and presented soluble antigens to initiate an immune response.

"The macrophage was regarded as a good system to study immune response initiation," says Steinman, "because they were thought to present intact antigen." But he found that the macrophages didn't present whole antigen; they simply degraded it (1). Steinman decided he'd better look beyond these cells.

Ralph M. Steinman

A new cell makes its mark

Leaving macrophages behind, Steinman turned to a mixture of cells from the mouse spleen. Suspensions of cells from this organ, which is one of the sites where immune responses get started, had been recently shown to induce antibody responses against sheep red blood cells (2). This was the first in vitro demonstration of a primary antibody response. But lymphocytes purified from the spleen could not bring about these responses unless they were mingled with a population of so-called "accessory" cells, a portion of which were macrophages.

This finding prompted Steinman to perform a very simple experiment. "I did something that evidently had not been done before," he says. He looked at these accessory cells in the culture dish. Thanks to his newly acquired skills in phase–contrast light microscopy, Steinman experienced a "Eureka!" moment. He found, mixed in with the macrophages, star-shaped cells unlike any immune cell seen before. When they crawled, the newcomers extended and retracted their radial

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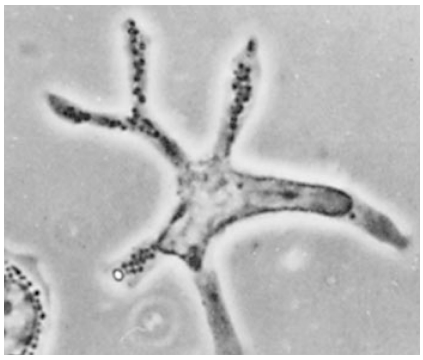

A phase–contrast image shows a dendritic cell.

branches. Since they were not phagocytic, they were lighter than macrophages on density gradients. As a result, recalls Steinman, "we could separate them cleanly from macrophages, even though this was before the days of monoclonal antibodies."

These cells were not just a culture dish artifact; Steinman found them in mouse peripheral lymphoid organs. And unlike the more populous macrophages, these cells did not take up antigens. Steinman and Cohn were convinced that a new cell type had arrived in the immune world—they christened them dendritic cells and described their physical and physiological features in two papers in the *Journal of Experimental Medicine* (3, 4).

Form finds function

The papers generated a spirited debate about whether these cells were a new class of white blood cells or merely an artifact. Many opponents thought they were too rare to make a difference. For believer Steinman, however, the next step was to test whether the DCs were the missing link between recognition and immune response. He designed a protocol to purify DCs from mouse spleen fractions that Michel Nussenzweig, one of Steinman's first graduate students, calls "a cumbersome, onerous task" that yielded only a tiny number of DCs. Nussenzweig made the enrichment process easier when he developed a DC-specific antibody a few years later (5). Wesley Van Voorhis, another graduate student, later used the same protocol to isolate DCs from human blood (6).

Using the enriched population, Steinman found that these cells expressed very high levels of a surface antigen called MHC class II, which was later shown to be an antigen-presenting molecule. Steinman tested whether DCs might be better than known antigen-presenting cells (APCs) at initiating immune responses. He and his then technician, Maggi Pack, added DCs or known APCs to a mixture of lymphocytes from two different donors—an assay known as a mixed leukocyte reaction (MLR). They found that the DCs were an impressive 100-fold better at activating T cells in the mix than were macrophages or B cells (7). "I was shocked by the numbers," says Pack, "but Ralph had already moved on to designing the next experiment." Van Voorhis found that the same was true for human DCs. "We presented our findings at a FASEB meeting and angered a lot of people," he recalls. "They were so attached to the idea that human monocytes were the best antigen presenters."

The assay proved that DCs presented their own antigens to T cells. But to prove that DCs were relevant physiologically, the team had to show that DCs could pick up external antigens, process and present them, and thereby activate lymphocytes. To do so, Nussenzweig coated one set of cells with antigen. He then added these cells to a mixture of DCs or other APCs. Only the DCs captured the antigen and presented it to T cells, which then became cytotoxic. This was the first demonstration that DCs could prompt $CD8^+$ T cell–mediated immunity (8).

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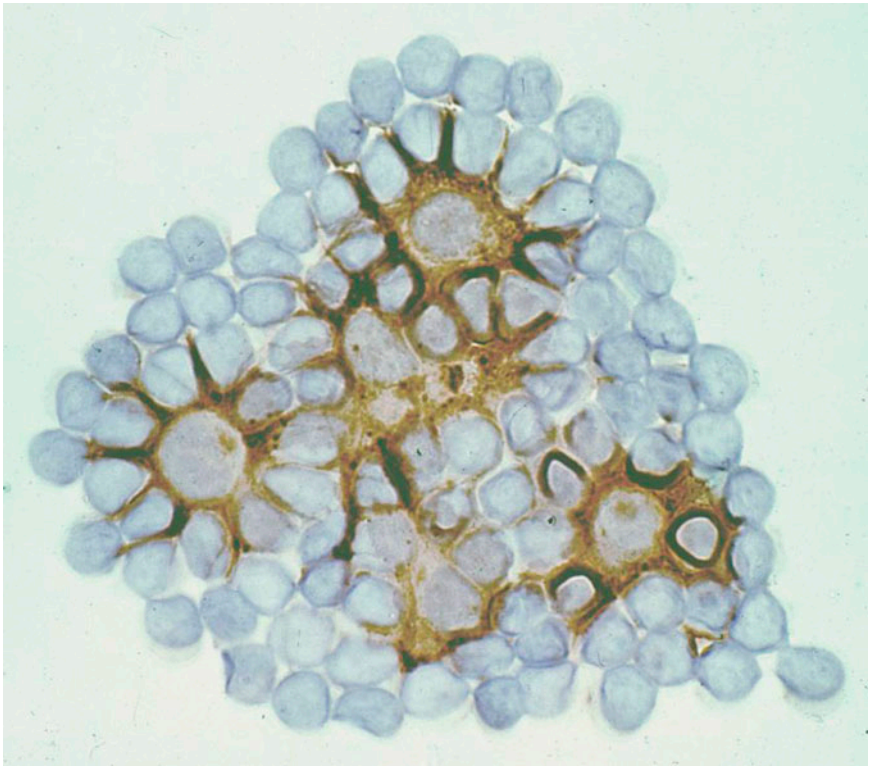
Kayo Inaba, a postdoc who had just arrived in the lab, showed that DCs also induced the development of CD4, "helper" T cells that then initiated potent antibody responses (9). DCs were now emerging as the command center



COURTESY OF NIKOLAUS ROMANI

Dendritic cells (brown) swarm around T cells (blue) to present them with antigen.

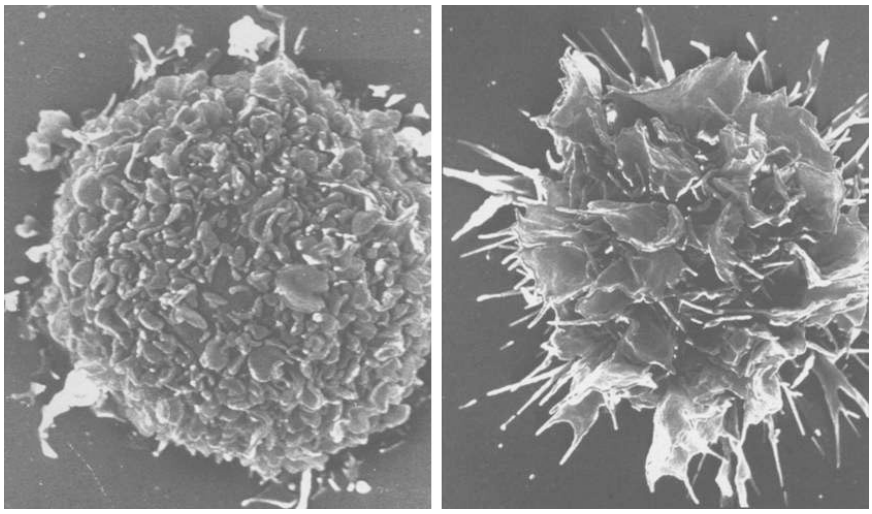
of the immune response operation. But Steinman admits, "we were still thinking of DCs only as cells that instigated a primary immune response. What we didn't appreciate was that there were many different facets of the immune response that DCs could control."

DCs go global

DCs had so far been identified in the spleen and in the T cell areas of Peyers patches. A good deal of this work had been done in Steinman's lab in New York. But around 1980, DCs began to make their international debut. Robert Lechler and Richard Batchelor at Hammersmith Hospital (London) recapitulated Nussenzweig's MLR experiment in vivo by showing that DCs injected into rats induced the rejection of foreign kidney grafts (10).

The DCs also began to show up in other areas of the immune system, including the lymph system. The identification of DCs in rabbit and rat lymph vessels, by Brigette Balfour, Gordon MacPhearson, Stella Knight, and others in England, and Hemmo Drexhage in the Netherlands, introduced the idea that these cells were a roving surveillance network (11, 12). Steinman proposed that DCs were picking up antigen in peripheral tissues and then migrating to lymphoid organs to start the immune response. This idea would explain why immune responses could also begin in the draining lymph nodes nearest the site of antigen deposition.

To test the idea, Steinman's team began to look for DCs in peripheral tissues. Austrian scientists Gerold Schuler and Nikolaus Romani, who had been studying antigen presentation in the skin, arrived in Steinman's lab to investigate whether skin Langerhans cells (LCs) were a form of DCs. The duo proved this link and made a second tantalizing discovery: DC maturation. Schuler found that LCs could pick up antigen but did not stimulate T cells unless they were first cultured with the cytokine GM-CSF (13). Romani found that the GM-CSF–"matured" cells could no longer capture antigen but were adept at stimulating T cells (14). Antigen capture was thus distinguished from immune responsiveness.



COURTESY OF GEROLD SCHULER AND NIKOLAUS ROMANI

Immature dendritic cells (left) that capture antigen extend processes and mature into APCs (right).

Building up the DCs

The biggest hurdle to DC research thus far was their limited availability—monoclonal antibodies and cell sorters easily sifted DCs from tissue suspensions, but DCs were a relatively rare population to begin with. "People weren't even convinced that these were cells with a separate pathway of differentiation," recalls Steinman. "Most people weren't making DCs to test them," he says. "Only those who took the trouble were impressed by their potency."

> **Steinman's response to all the theorizing: "Just do the experiment!"**

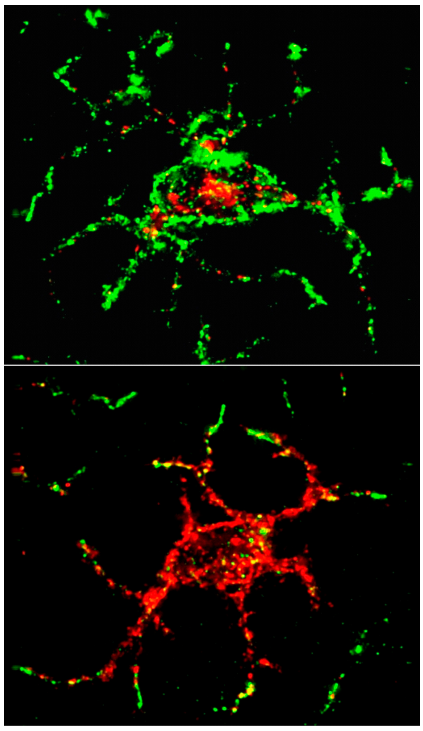
But in the early 1990s, three teams, including Steinman's group, grew DCs from different sources. Steinman and his team discovered that DCs could be grown from GM-CSF–treated blood and bone marrow progenitor cells. Teams led by Jacques Banchereau at Schering Plough in France and Antonio Lanzavecchia at the Basel Institute in Switzerland found that DCs could be grown from $CD34^+$ hematopoeitic progenitor cells from humans and differentiated from human blood monocytes (15–17).

Enhancement versus tolerance

With DCs now readily available, Steinman began to test their mettle in vivo. Immature DCs that were cultured with antigens and injected into mice jump-started immune responses without help from adjuvants such as alum or Freund's (18). Steinman dubbed these cells "nature's adjuvants."

Other groups started to exploit this adjuvant-like nature of DCs to boost the immune systems of cancer patients. Monocytes were extracted from patients, differentiated into DCs, and loaded with tumor antigens. When these DC "vaccines" were reinfused into patients, some tumors were destroyed. But the failure of injected DCs to migrate properly and establish themselves in lymphoid tissues prevented this approach from being more successful.

Madhav Dhodapkar, a Rockefeller scientist who is using DCs to boost human immunity to multiple myeloma tumors, benefits regularly from Steinman's input. "He's such a great supporter of human work," says Dhodapkar. But he cautions that this research area is still very much in its early stages. "Many groups have shown that this approach has immune efficacy and can be carried out safely. But it is still an evolving science, and there is a lot left to do to optimize this process."



COURTESY OF JULIANA IDOYAGA AND IRA MELLMAN

MHC class II proteins (red) inside immature Langerhans cells (green) are expressed on the surface when the cells mature (bottom).

Steinman himself is less than pleased with the speed with which this line of work has evolved. "This idea [of DC immunotherapy] has progressed very slowly," he laments, "even though immunology clearly needs to be explored in depth to help cancer patients." He blames poor funding and badly coordinated studies for the slow progress of DCs from the lab bench to the intravenous drip.

A decade after DCs were identified as powerful immune enhancers, Steinman and Nussenzweig—now also a faculty member at Rockefeller—added a new twist to the DC tale. They found that when antigens were captured by DCs that were unaltered either by culturing or inflammation, the DCs killed T cells instead of activating them, thus establishing tolerance (19). Inaba, who continues to collaborate with Steinman, simultaneously showed that immature DCs that corral dying infected cells also fail to activate T cells and induce tolerance (20). Steinman's group later found that DCs also induced the expansion of regulatory T cells (21). This work suggests that the DC network is wired to steer the body away from self-reactivity when danger is absent.

The CDs of DCs

For Steinman, the question of how antigens are delivered to start an immune response has evolved into how DCs control the quality of the immune response. He and his group are currently trying to target antigens directly to the DC receptors that take them in—a process shown to enhance immunity. CD205 (also known as DEC-205) is the primary antigen-binding receptor in many DC subsets. Coupling an anti-CD205 monoclonal antibody to an antigen increases the antigen's presentation by ~100 fold (22).

Steinman also wonders how maturation signals tweak the DCs to modify immune responses, for example, to guide the development of T helper (Th)-cells into Th1 or Th2 subsets. "We need to understand what information a T cell needs to receive from a DC before it starts responding along a particular path," he says. As DCs vary their expression of costimulatory receptors such as CD40, CD70, and CD80/86 depending on the maturation stimuli, the strength and quality of the stimulation signal they impart probably help determine the function of the responding cell.

> **"It took almost ten years for people to accept that DCs and their immune potency were for real. But history proved [Steinman] right."**
> **— Wesley Van Voorhis**

Ending credits

Steinman credits the progress and interest that DC biology has enjoyed to fellow DC enthusiasts in his own lab and around the globe, endearing him to his coworkers and peers. Says former graduate student Josh Metlay, "Ralph's way of pursuing an idea was not to put one step in front of another; he liked to get people to approach the same problem from different angles." Others recall his eagerness to get to the scientific task at hand when ideas were bandied about during lab meetings. Steinman's response to all the theorizing: "Just do the experiment!"

Ralph Steinman's research career, which began with finding a new cell, resulted in the founding of an entire research field. His colleagues in the early years recount the uphill task he faced. "Ralph was the lone voice in the desert," says Van Voorhis. "It took almost ten years for people to accept that DCs and their immune potency were for real. But history proved him right."

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