

RALPH STEINMAN AND THE DISCOVERY OF DENDRITIC CELLS

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by

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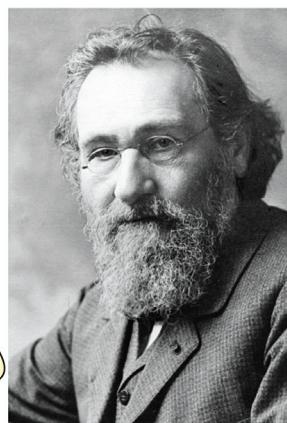
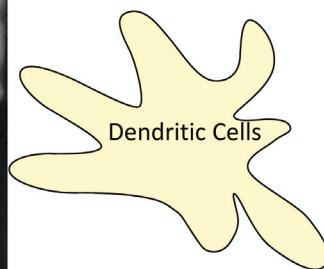
In science, it is a rare event for one individual to make a discovery that opens a new scientific field, work at the forefront of its research for forty years, and live to see his endeavors transformed into novel medical interventions. Ralph Steinman was such an individual. His discovery of dendritic cells changed immunology.

HISTORICAL BACKGROUND

The dendritic cell discovery was a key breakthrough in immunology because it brought together the work of Paul Ehrlich and Ilya Metchnikov, who shared the Nobel Prize in Physiology or Medicine in 1908 for their work on immunity (Fig. 1).



Paul Ehrlich
Adaptive Immunity



Ilya Ilyich Metchnikov
Innate Immunity

Figure 1. Dendritic cells connect the adaptive and innate immune systems featured in the work of Paul Ehrlich and Ilya Metchnikov, who were awarded the Nobel Prize in Physiology or Medicine in 1908.

Ehrlich focused on adaptive immunity and the exquisite specificity of the serologic response by the immune system to produce antitoxins, each of which was unique. He suggested a model whereby cells with receptors for the toxins would recognize the toxins and then release excess toxin receptors into circulation. Ehrlich was also the first to frame the problem of diversity: if the immune system can respond to any invading organism and destroy it, how does it know the difference between self and non-self antigens so that it prevents attacks on self, a condition he called *horror autotoxicus* known today as autoimmunity.

Metchnikov, on the other hand, focused on innate immunity. He discovered phagocytosis in starfish larvae and proposed there are innate cells, particularly macrophages, that internalize and kill microbes. There was nothing for cells to learn because the immune system provided an instinctive response for phagocytes to destroy pathogens.

How the adaptive and innate immunity are related was entirely not clear at the time, but we now know dendritic cells are the missing link that connects Ehrlich and Metchnikov. Dendritic cells are a part of the innate system and they orchestrate adaptive immunity.

Steinman learned about immunity at Harvard Medical School and especially during courses he took in cell biology during the 1960s (Fig. 2). Those were heady times for immunology. The immune system was being linked to a number of mysterious diseases like lupus and arthritis, which are due to *horror autotoxicus*, and there were successful new vaccines against infectious diseases that were making an enormous difference in public health.

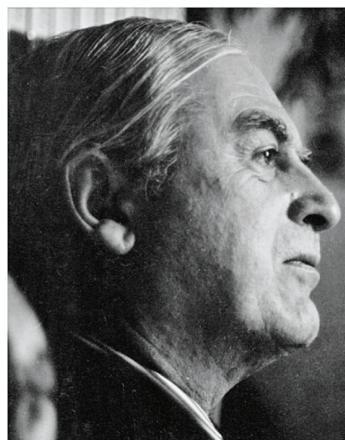


Figure 2. Ralph Steinman, far right, as a medical student at Harvard in the 1960s when he started to learn about the immune system.

Steinman was particularly interested in the work of Macfarlane Burnet and Peter Medawar, who were awarded the Nobel Prize in Medicine or Physiology in 1960 for their work on acquired immune tolerance (Fig. 3). Burnet, in particular, had published several lectures in which he tried to establish a theoretical framework to explain Ehrlich's dilemma about how the immune system distinguishes self from non-self. Burnet outlined the three cardinal features of immunity as Specificity, Diversity, and Memory. Specificity, going back to Ehrlich, meant immune responses that recognize one toxin do not cross-react with a different toxin. Diversity, a concept championed in the 1930s at The Rockefeller Institute by Nobel laureate Karl Landsteiner, referred to the finding that even substances that do not exist in nature can induce an immune response, thus leading to the conclusion that the immune system can recognize any invading organism, pathogen, or toxin. Memory, going back to Edward Jenner and smallpox in the 18th century, is a basic property of vaccines; once the immune system sees a toxin and it reappears, the system recognizes it and can respond faster and better.



Macfarlane Burnet
[Clonal Selection](#)



Peter Medawar
[Immune Tolerance](#)

Figure 3. Macfarlane Burnet and Peter Medawar, who were awarded the Nobel Prize in Physiology or Medicine in 1960 for their work on immunological tolerance.

To account for these properties, Burnet (Burnet, 1957; Burnet, 1958) contemporaneously with David Talmage (Talmage, 1957) suggested that the immune system is composed of clones of cells, each expressing a unique receptor (Fig. 4). Specificity would come from the receptor expressed on these cells (as hypothesized by Ehrlich) and would be unique for each cell. Diversity would be accounted for by the large number of individual clones emerging during development. According to Burnet's theory, when antigen comes into the system, it selects the right clone and that clone is expanded and memory is induced. Nobel laureate Joshua Lederberg added to this idea by suggesting that any self-reactive clones that arise during development

would be deleted during encounters with self antigens (Lederberg, 1959). Finally, immune responses begin when a pathogen or antigen enters the system and causes expansion of the specific clone, whereas memory involves simply more cells that are specific for the pathogen so there is a faster and better response the second time around.

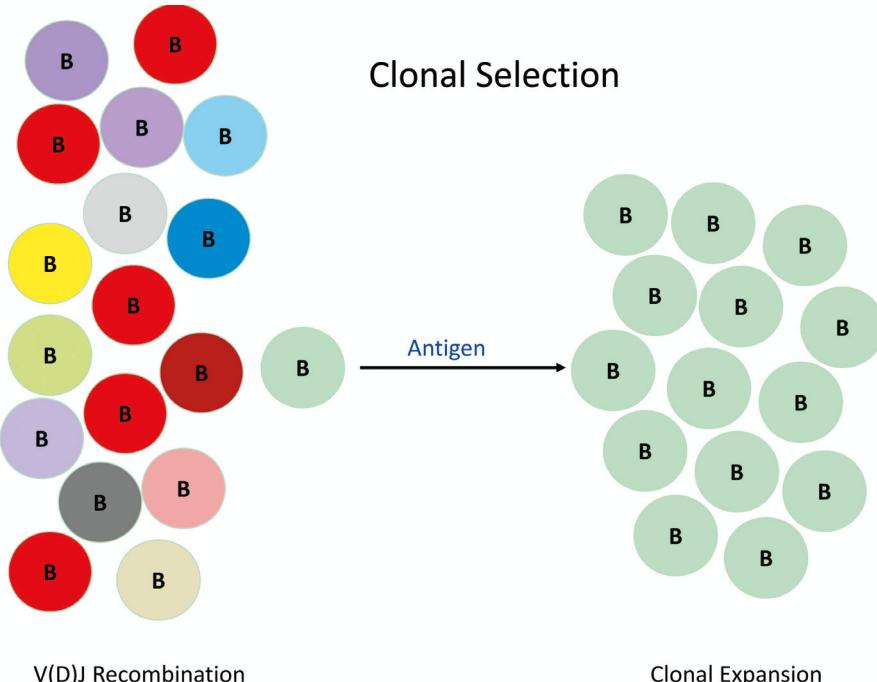


Figure 4. The clonal selection theory. Clones of lymphocytes, each with a different unique receptor (represented by a different color), are present before antigen is introduced into the system. The antigen selects the clone with the best cognate receptor for expansion.

The most intriguing problem in immunology during the 1960s was how to create sufficient diversity to account for adaptive immunity. But neither Burnet nor Steinman was interested in this problem. Steinman wanted to learn how an immune response begins, and he believed that understanding this feature of immunity would make it possible to regulate immune responses, both to prevent autoimmunity and to create vaccines.

DISCOVERY

In 1970, Steinman joined physician-scientists Zanvil A. Cohn and James G. Hirsch in The Rockefeller University laboratory of René Dubos, a microbiologist (Fig. 5) who was a disciple of Oswald T. Avery, in whose Rockefeller laboratory he discovered the natural antibiotic gramicidin in 1939 (Hirsch and Moberg, 1989; Moberg and Steinman, 2003, 2009). The Dubos laboratory had evolved from studying microbes to focus on interactions of the host with pathogens. Metchnikov became the hero for Cohn and Hirsch.



Zanvil Cohn

René Dubos

James Hirsch

Figure 5. Ralph Steinman's mentors at The Rockefeller University.

An important breakthrough in immunology in 1967 was the development of a method to study specific immune responses *in vitro*. The system developed by Robert Mishell and Richard Dutton involved mixing antigens with lymphocytes and measuring antibody responses (Mishell and Dutton, 1967). An unexpected finding was that lymphocytes alone were not sufficient to produce immune responses and that accessory cells were required to initiate immunity (Fig. 6). Although the nature of the accessory cells was unknown, it was a cell that was adherent to glass and its role was to present antigen to lymphocytes.

Mouse Spleen Cell Mishell-Dutton Cultures: the Need for Accessory Cells

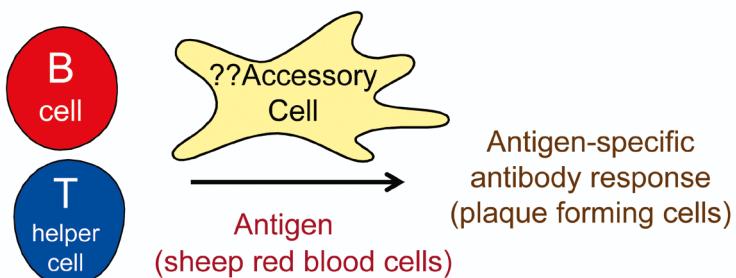


Figure 6. The diagram summarizes the observation that T and B cells are not sufficient to induce antibody immune responses *in vitro* and that reaction requires an accessory cell.

Following his interests in initiation of immunity, Steinman worked closely with Cohn to look for the accessory cells. Most workers in the field at the

time believed the accessory cell was a macrophage. In vivo studies by other laboratories showed cells looking like macrophages were associated with antigens in lymph nodes (Nossal et al., 1968), and when macrophages were loaded with antigens, they induced immune responses (Unanue and Cerottini, 1970).

However, Cohn had spent his career studying macrophages and had shown with Barbara Ehrenreich that when antigens such as albumin were captured by macrophages, they were degraded to amino acids (Ehrenreich and Cohn, 1967). Steinman's first assignment as a postdoctoral fellow in the Cohn laboratory was to explore that finding. He developed a method using horseradish peroxidase and horseradish peroxidase-immune complexes to see whether this protein could be detected on macrophage surfaces where it would be displayed to lymphocytes that would induce immunity. While this method allowed quantitative measurements and ultrastructural visualization of ingested antigens, Steinman failed to find antigen uptake or retention by macrophages. Instead, he made an important discovery in cell biology when he found that the uptake of horseradish peroxidase was so rapid that he and Cohn concluded that macrophages must be constantly recycling their membranes (Steinman and Cohn, 1972a, b).

To look for a different accessory cell, Steinman and Cohn decided to study adherent cells from mouse spleen instead of from the peritoneal cavity. They were fortunate to have help from their Rockefeller colleagues, two cell biologists who won the 1974 Nobel Prize in Physiology or Medicine for their discoveries of cell structure and function (Fig. 7). George Palade's laboratory pioneered fixation and electron microscopy of cells, tools that Steinman used to identify dendritic cells. Christian de Duve's laboratory was expert in centrifugation methods to separate subcellular components, a technique that Steinman adapted in devising a scheme to purify dendritic cells.



Figure 7. George Palade and Christian de Duve, who were awarded the Nobel Prize in Physiology or Medicine in 1974 for their work in cell biology, were instrumental in developing techniques used by Ralph Steinman to characterize and purify dendritic cells (DC). Courtesy of The Rockefeller Archive Center.

What Steinman and Cohn found in 1973 when they looked through a phase contrast microscope was a different cell (Steinman and Cohn, 1973). It had dendritic processes but no prominent phagocytic vacuoles (Fig. 8). This is the discovery that was honored with the 2011 Nobel Prize in Physiology or Medicine. Steinman and Cohn used electron microscopy to confirm their phase contrast observations and saw features that were distinct from typical macrophages and monocytes (Steinman and Cohn, 1973). The cells were elongated with tree-like processes that were constantly forming and retracting. Steinman named them dendritic cells, from the Greek word *dendreon* for tree. The dendritic cells also had a few, small lysosomes and lacked the typical membrane ruffling seen in phagocytes. With micro-cinematography they observed dendritic cell behavior that was dynamic and distinct from macrophages that were sedentary. Using the best microscopic and ultra-structural techniques available, they were confident they had found a novel cell.

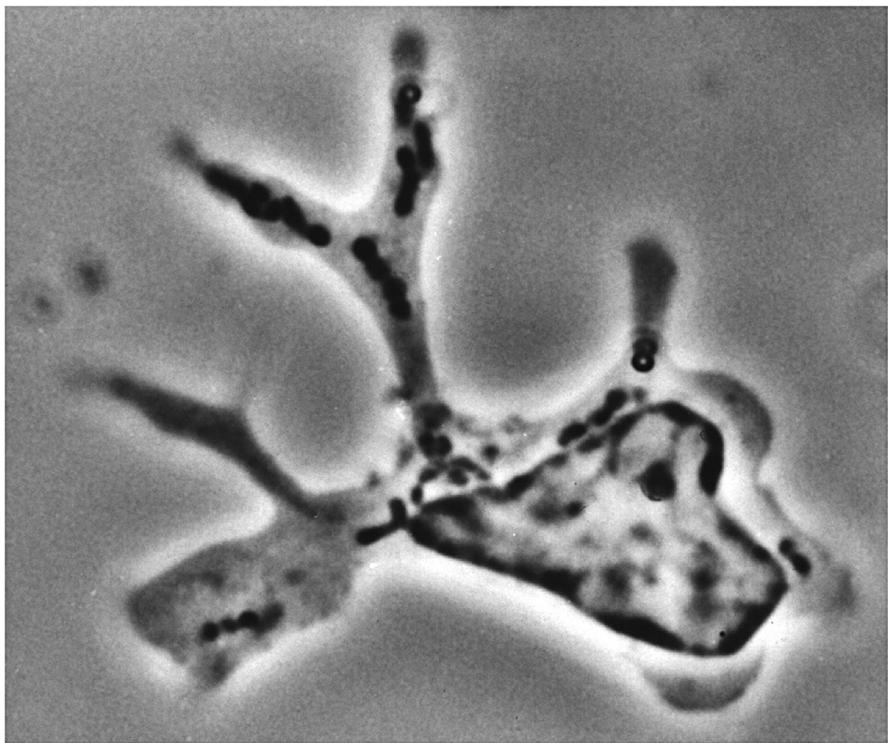


Figure 8. Phase contrast micrograph of a dendritic cell (Steinman and Cohn, 1973).

PURIFICATION

Steinman intuited that the newly discovered cell was the accessory cell, but he needed to devise a method to purify the cells before he could test this idea (Steinman and Cohn, 1974). At the time there were no easy procedures for cell purification and it took him a couple years to develop a technique based

on physical properties of dendritic cells (Fig. 9). A suspension of spleen cells was subjected to de Duve's methods of density gradient centrifugation in a column of bovine serum albumin, on which semi-purified dendritic cells rose to the top and small lymphocytes went to the bottom. Steinman then placed this low-density fraction on glass for an hour and then delicately washed away all except the adherent dendritic cells and macrophages. Following an overnight culture, the dendritic cells detached from the glass. Steinman then devised a method for removing the lingering macrophages by adding antibody-coated sheep red blood cells that formed rosettes by virtue of their Fc receptor expression. The rosetted macrophages went to the bottom of a second gradient centrifugation and the dendritic cells were collected from the light interphase. Unfortunately, the yield of dendritic cells by this method was poor and there were never enough cells to do all the experiments that Steinman wanted to do. Moreover, it was difficult for others to replicate this tedious procedure. In addition, the techniques were familiar to cell biologists but not to immunologists. As a result, Steinman and his students had dendritic cell studies all to themselves for the next fifteen years.

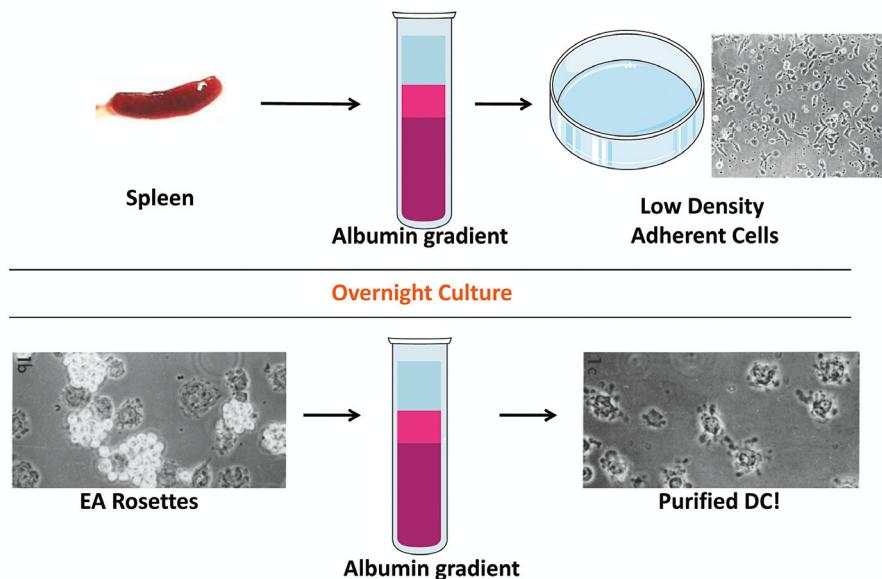


Figure 9. Original method for dendritic cell purification. Spleen cell suspensions are fractionated on albumin density gradients and low density cells placed on glass to capture adherent cells. Following overnight culture, cells that come off the glass are incubated with antibody coated erythrocytes (EA). The mixture is separated by a second round of albumin gradient centrifugation to obtain purified dendritic cells.

MIXED LEUKOCYTE REACTION

To demonstrate that dendritic cells were functionally unique, Steinman and Maggi Witmer-Pack used the mixed leukocyte reaction (MLR) (Fig. 10). This model had been used for tissue typing to predict whether a patient would

accept or reject a transplanted graft. In this experiment, dendritic cells from one mouse strain were mixed with T cells from another mismatched strain and the T cell proliferative response to the mismatched dendritic cells was measured. Steinman found that dendritic cells were nearly 100 times more potent in inducing the MLR than non-fractionated mixtures of spleen cells. Since the dendritic cells account for only 1–2% of all cells in the spleen, he concluded that dendritic cells were the key stimulators of this reaction. He also made the leap to suggest that dendritic cells might be the accessory cells (Steinman and Witmer, 1978).

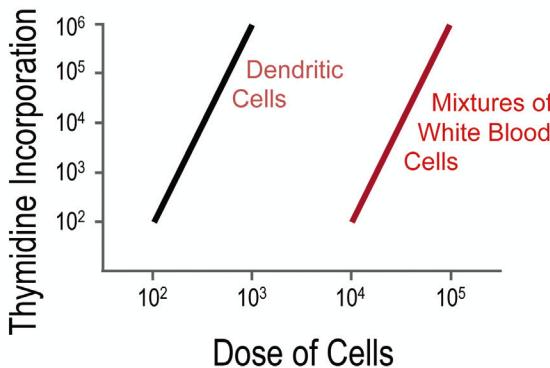


Figure 10. Dendritic cells are potent stimulators of the mixed leukocyte reaction (Steinman and Witmer, 1978). The graph shows a comparison of the stimulatory activity of dendritic cells and unfractionated spleen cells. The photograph shows Maggi Witmer-Pack, who worked with Ralph Steinman on these experiments.

Nevertheless, most immunologists did not accept this conclusion, as William Paul pointed out in his commentary in Cell on the 2011 Nobel Prize in Physiology and Medicine: “This report was initially received with some skepticism, based on the widely held view that the major antigen presenting cells were the far more numerous macrophages and on the uncertainty that many immunologists had about the assay that Steinman and Cohn used to establish the function of their dendritic cells (Paul, 2011).” In other words, the MLR was not thought to be a typical adaptive immune response but more like a spontaneous, innate response. Also, the precise nature of the antigen and the reacting cells were not well defined.



Michel Bodma Maggi Ralph
Nussenzweig Gutchinov Pack Steinman 1980

DENDRITIC CELLS ARE ACCESSORY CELLS FOR THE
DEVELOPMENT OF ANTI-TRINITROPHENYL CYTOTOXIC T
LYMPHOCYTES*

By MICHEL C. NUSSENZWEIG, RALPH M. STEINMAN,‡ BODMA GUTCHINOV,
AND ZANVIL A. COHN

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"DC are the critical accessory cells, whereas macrophages regardless of source or expression of Ia (MHC II) are without significant activity."

Figure 11. Investigators who demonstrated that dendritic cells are antigen presenting cells and the title of that 1980 paper (Nussenzweig et al., 1980).

ANTIGEN PRESENTATION

By 1978, an experimental system was needed to measure a more typical antigen-specific adaptive response and show that dendritic cells are antigen presenting cells. Steinman's first graduate student, Michel Nussenzweig, developed such an assay based on Karl Landsteiner's pioneering work that utilized haptens as the antigens to elicit responses (Fig. 11). The assay adapted to dendritic cells involved modifying cells with the nitrophenyl moiety and measuring the development of cytotoxic or killer T lymphocytes. Dendritic cell responses were compared to those of macrophages and other accessory cells (Nussenzweig et al., 1980). Results of these experiments showed that "dendritic cells are the critical accessory cells whereas macrophages regardless of source or Ia (MHCII) are without significant activity." With these experiments Steinman and Nussenzweig established the important principle that dendritic cells present antigen to T cells to initiate immunity.

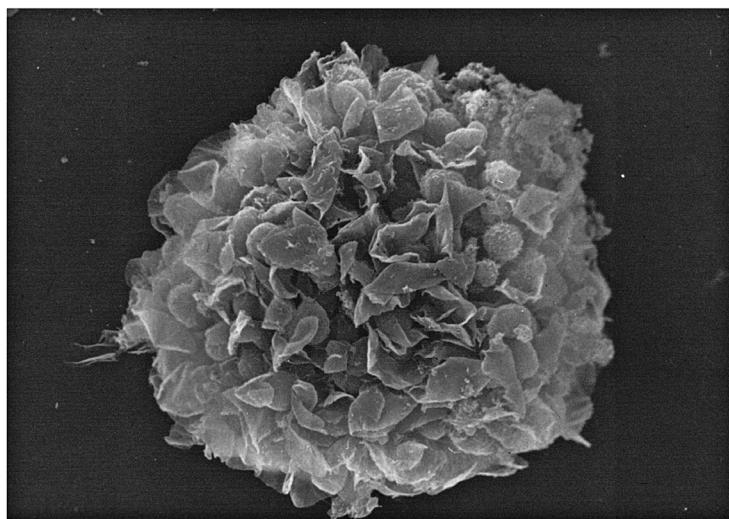


Figure 12. Scanning electron micrograph of a cluster of T cells and dendritic cells illustrates their large membrane projections. Courtesy of Dr. Gilla Kaplan.

By 1980, it was clear that dendritic cells had unique morphological features (Fig. 12), behaved differently, had specific physical properties that allowed their purification, and had remarkable T cell stimulatory activity *in vitro*. However, methods did not exist to eliminate dendritic cells from cell mixtures or to locate them in the organism. Moreover, since there were no molecular markers for dendritic cells, it was still difficult to convince anyone that they were really a distinct cell type. The first step in that direction was the development by Michel Nussenzweig of the monoclonal antibody 33D1 that is specific for the major dendritic cell subset in spleen (Nussenzweig et al., 1982). It was used to visualize dendritic cells in tissue sections and to deplete dendritic cells from complex mixtures of cells. T cell responses were decreased when dendritic cells were depleted from cell mixtures of cells (Fig. 13, (Steinman et al., 1983)). Although only 1% of the cells were killed, there was a very significant decrease in the ability of spleen cell mixtures to stimulate the mixed leukocyte reaction. Therefore, the 33D1 monoclonal antibody became a molecule that distinguished dendritic cells from other cells and a tool that could determine their role in cell mixtures.

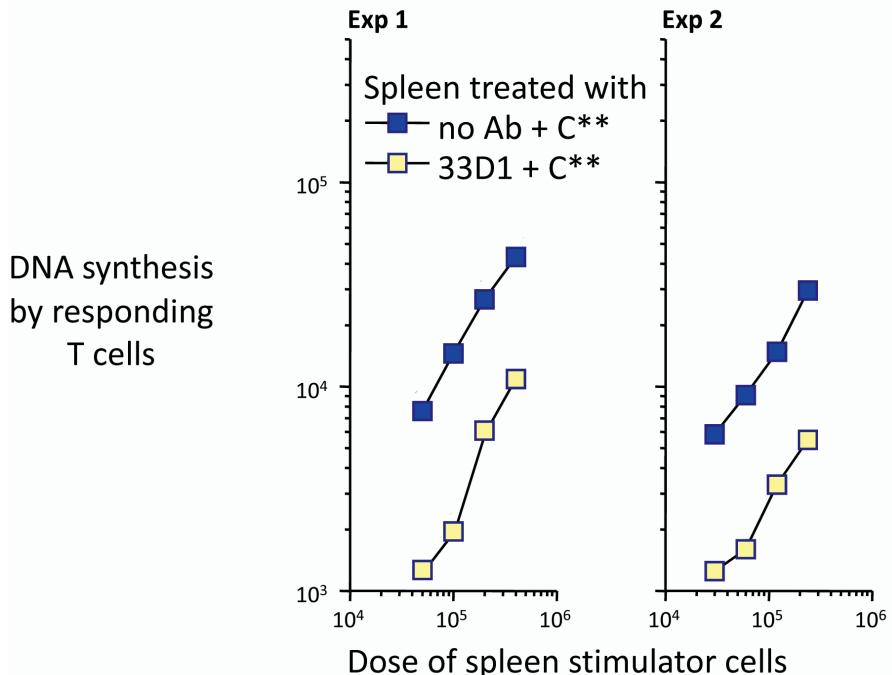


Figure 13. Graph shows results of mixed leukocyte reactions in which mixtures of spleen cells were treated with anti-33D1 monoclonal antibody to eliminate dendritic cells carrying this surface antigen and compared to untreated controls (Steinman et al., 1983). The Y axis displays DNA synthesis as measured by H3Thymidine incorporation and the X axis shows the dose of stimulator cells added to the culture. C** refers to Complement. Ab refers to antibody.

Steinman had two early collaborators who added important contributions to establishing the antigen presentation properties of dendritic cells (Fig. 14). Wes Van Voorhis, an MD-PhD student in the laboratory, was the first to study human dendritic cells; he showed they exist in blood, are distinct from blood monocytes, and are antigen presenting cells (Van Voorhis et al., 1982).

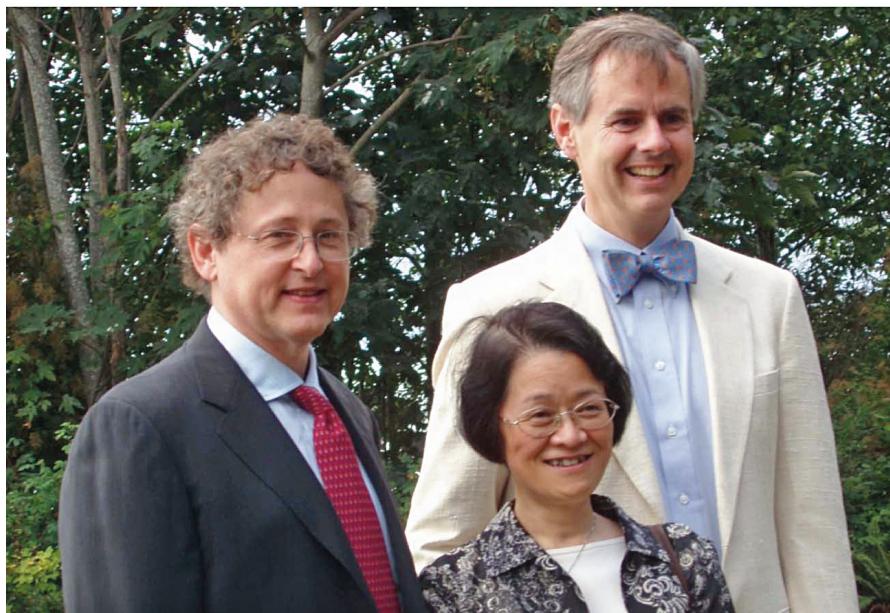


Figure 14. Ralph Steinman's early collaborators. From left to right: Michel C. Nussenzweig, Kayo Inaba, and Wesley Van Voorhis.

Kayo Inaba, who had shown independently in Japan that a non-macrophage in spleen could function as an accessory cell (Inaba et al., 1981), continued the work on antigen presentation in a variety of different systems, most notably the Mishell-Dutton system (Inaba et al., 1983a; Inaba and Steinman, 1985). When accessory functions of purified dendritic cells were compared to adherent cells or dendritic cell-depleted adherent cells, dendritic cells were found to be far superior antigen presenting cells compared to mixtures of adherent cells. Furthermore, depletion of dendritic cells from mixtures of cells using 33D1 led to a loss of accessory activity (Fig. 15) (Inaba et al., 1983a)

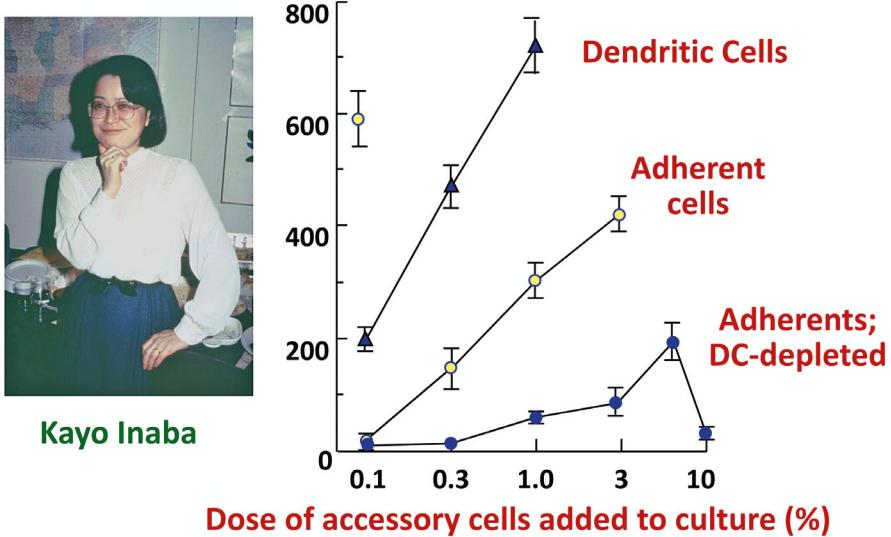


Figure 15. Kayo Inaba is shown on the left. On the right a plaque forming cell response in Mishell Dutton cultures where T and B cells are supplemented with increasing number of dendritic cells, mixtures of adherent cells or adherent cells depleted of dendritic cells that express the 33D1 antigen (Inaba and Steinman, 1985; Inaba et al., 1983b). Y axis shows the number of plaque forming cells and the X axis the number of accessory cells added to the cultures.

Based on their work at the The Rockefeller University, Nussenzweig, Inaba, and Steinman put forward a model to explain how dendritic cells capture and process the antigen and present it to T cells (Fig. 16). The interaction between T cells and dendritic cells is a cognate interaction in which T cells recognize the specific antigen being displayed by the dendritic cell. Most importantly, the interaction leads to T cell activation and allows them to perform their various effector functions. Under some conditions, the activated T cell stimulates cognate B cells to produce antibody (Fig. 16).

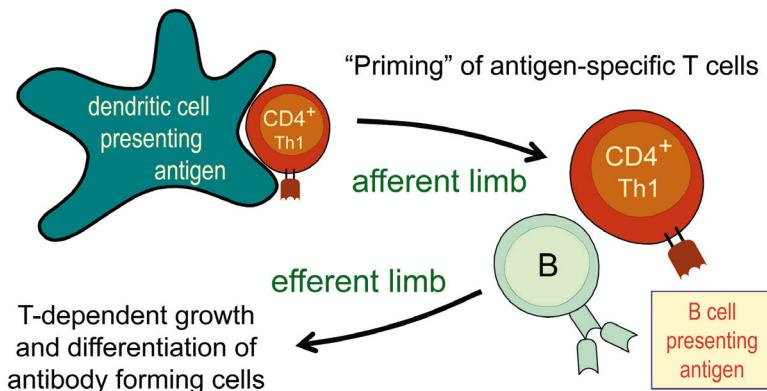


Figure 16. A model put forward by Ralph Steinman and his colleagues to explain the role of dendritic cells in inducing B cell antibody responses. Dendritic cells present antigen to and activate T cells which then interact with antigen specific B cells, by virtue of antigen presentation by the B cells.

Inaba and Steinman did an additional ground breaking experiment on anti-tumor and anti-viral activities of dendritic cells. When they removed dendritic cells from mice, loaded them with antigen, and re-infused them into mice, there was strong protective immunity (Fig. 17, (Inaba et al., 1990)). This finding established the significant principle that antigen presentation of dendritic cells in vivo could be the basis for immunotherapy experiments.

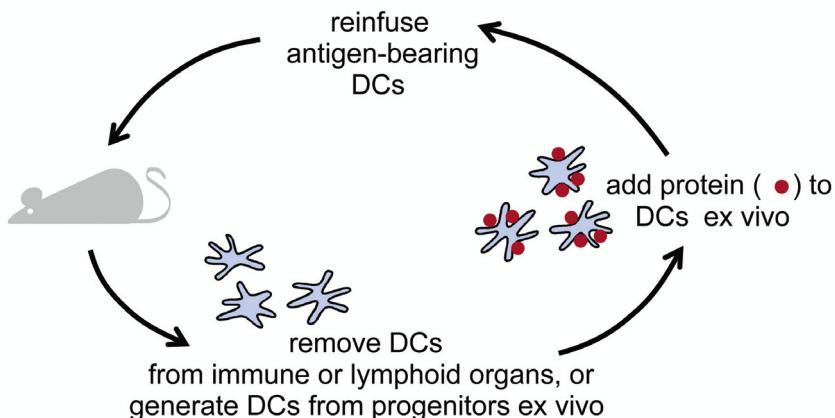


Figure 17. Diagram of the protocol for showing how antigen-loaded dendritic cells can be re-infused into mice to induce potent immune responses (Inaba et al., 1990).

Currently, immunotherapy is used in humans and is being tested by Steinman's clinical collaborators (Fig. 18). In this treatment dendritic cells are removed from the patient, expanded in culture, and loaded with the tumor antigen before being re-infused into the patient. This is the basis for the therapy of the first drug, Provenge, approved by the U.S. Food and Drug Administration to treat prostate cancer. This is also the basis for the therapy Steinman used to treat his own pancreatic cancer.

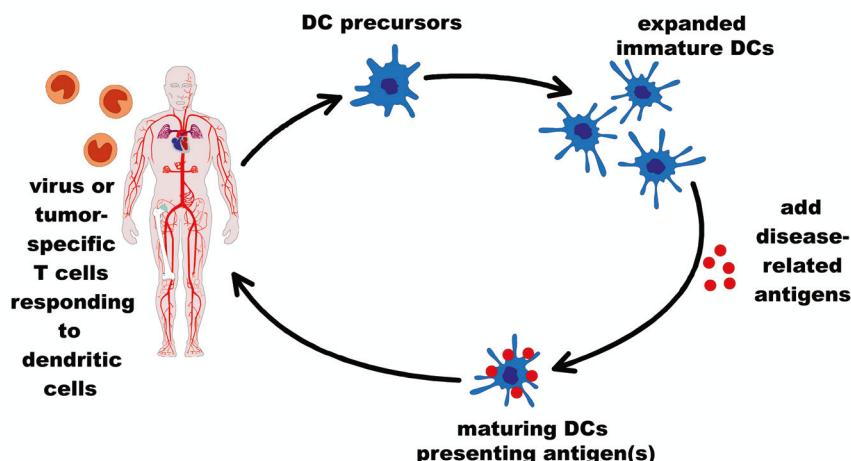


Figure 18. Diagram of the protocol for immunizing humans with antigen-loaded monocyte derived dendritic cells.

Together, these early experiments brought a new essential element to Burnet's theory concerning immune responses (Fig. 19). Burnet's idea remains that clones of lymphoid cells with specific receptors are selected to expand in response to the pathogen, but what is new is that the response depends on antigen capture and presentation by an innate cell. In this new model, the dendritic cell carries the antigen and selects the lymphocyte that is clonally expanded. This process, which was not anticipated by early immunologists, turns out to be an essential step in initiating immunity.

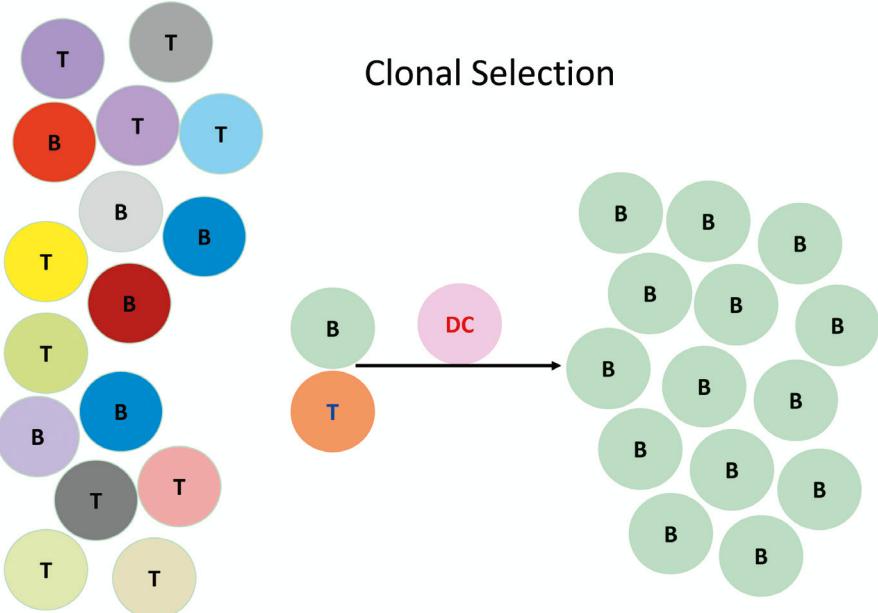


Figure 19. The role of dendritic cells in clonal selection. Antigen no longer activates the lymphocytes directly as originally proposed by Burnet and shown in Figure 4. Instead, antigen is presented to T lymphocytes or B cells by dendritic cells to initiate immunity.

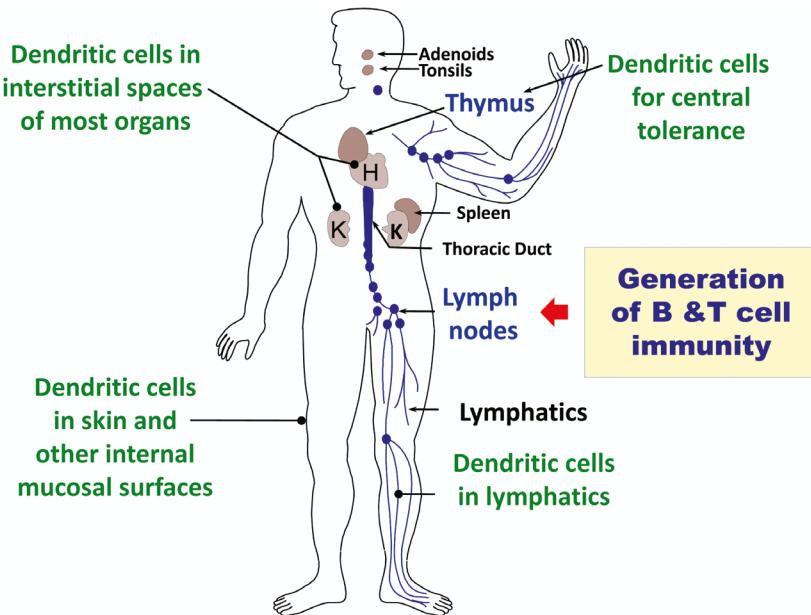


Figure 20. Diagram of the human body indicating the lymphoid organs and the location of dendritic cells in non-lymphoid organs. H refers to heart, K refers to kidneys.

Where are the dendritic cells located in the body (Fig. 20)? Soon after antibodies to dendritic cells became available, Steinman and his colleagues systematically investigated their location throughout the organism. The immune system, unlike the brain or the liver or heart, is a collection of cells that migrate through all the tissues in the organism. Dendritic cells are present at all the interfaces between the body and environment: airway epithelium (Fig. 21), skin, and mucosal surfaces. In other words, they are perfectly positioned to be sentinels and to capture antigens when and where they enter the organism.

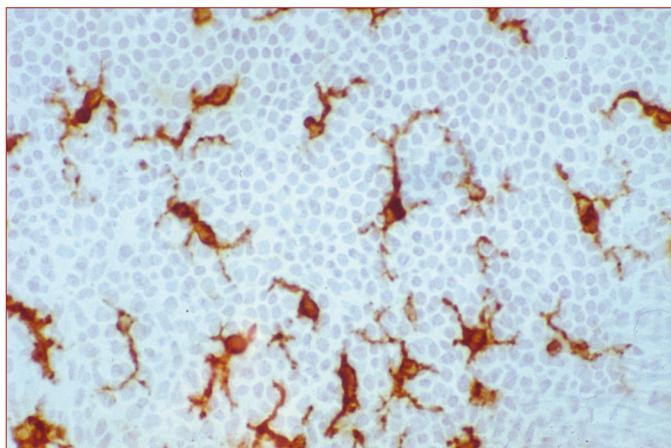


Figure 21. Micrograph showing dendritic cells in the airway epithelium. Courtesy of Dr. Patricia Holt.

Most importantly, collections of dendritic cells are found in lymphoid organs: lymph nodes (Fig. 22), spleen, tonsils, and thymus. In a movie made by 2-photon live imaging by Gabriel Victora, the dendritic cells in the T cell zone extend their processes throughout and create a dense network of cells (Movie #1). The T cells are migrating around and through this network in search of antigens on the dendritic cells (Movie #2). And when they meet an antigen on the dendritic cell, they stop, communicate, and receive the signal that activates their immune response (Movie #3). Again, the dendritic cells are in the right place where they interact with T cells in the adaptive immune system to initiate their immune response.

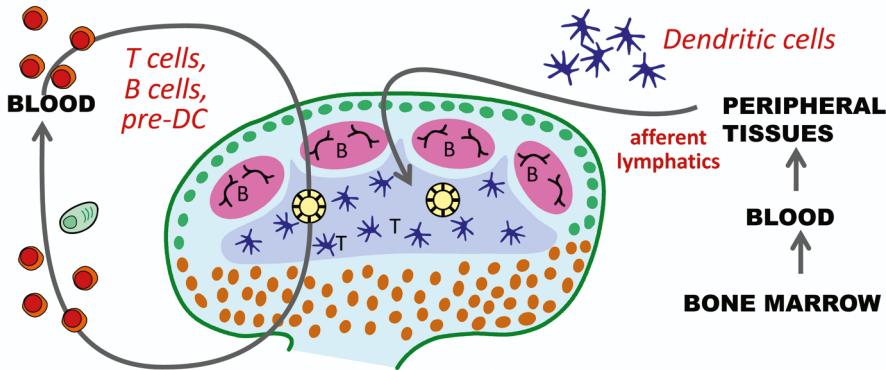


Figure 22. Diagram of a lymph node showing the position of dendritic cells in the T area. Dendritic cells arrive in the lymph node from the blood as pre-DC and from the tissues via the lymph. T and B cells circulate through the lymph nodes.

Thus, the histology showed that dendritic cells are positioned as sentinels in the innate immune system, as conceived by Metchnikov, and they are also positioned to connect with the adaptive immune system cells, as conceived by Ehrlich, to initiate the responses by effector cells.

Movie 1. Mouse lymph node with CD11c-YFP (Lindquist et al., 2004) labeled dendritic cells shown in yellow. Dendritic cells form a network in the T cell area of the node. Most cells remain in their position, and increase their surface area of antigen presentation with membrane extensions which are actively motile. Courtesy of Dr. Gabriel Victora.

Movie 2. Highlights the searching behavior displayed by the migrating blue fluorescent protein expressing T cells in a lymph node where dendritic cells are yellow labeled with CD11c-YFP.

Movie 3. Highlights T cell behavior upon meeting a dendritic cell presenting its cognate antigen. The T cell (fuchsia) remains in contact with the dendritic cell for a prolonged period of time.

DENDRITIC CELL MATURATION

Another important discovery made by Steinman and colleagues during the mid-1980s was that dendritic cells do not exist in just one state. In order to

initiate immune responses, they need to be activated by signals from pathogens or other activated immune cells. Steinman called this step maturation. Working with Gerold Schuler, a dermatologist, and Nikolaus Romani (Fig. 23) (Schuler and Steinman, 1985), Steinman found that immature dendritic cells are either poor stimulators or unable to induce immunity. However, once dendritic cells received activation signals, such as innate signals from Toll receptors, they matured, expressed high levels of MHC antigens, and became excellent antigen presenting cells (Fig. 24). Ira Mellman at Yale University working closely with Steinman uncovered the cellular basis of this phenomenon (Pierre et al., 1997). They showed that the switch between inactive and active states could in part be explained by re-distribution of MHC Class II molecules from lysosomes in immature dendritic cells to their cell surface during maturation where they would be recognized by T cells (Fig. 24).



Figure 23. Ralph Steinman's collaborators Nikolaus Romani (left) and Gerold Schuler (right).

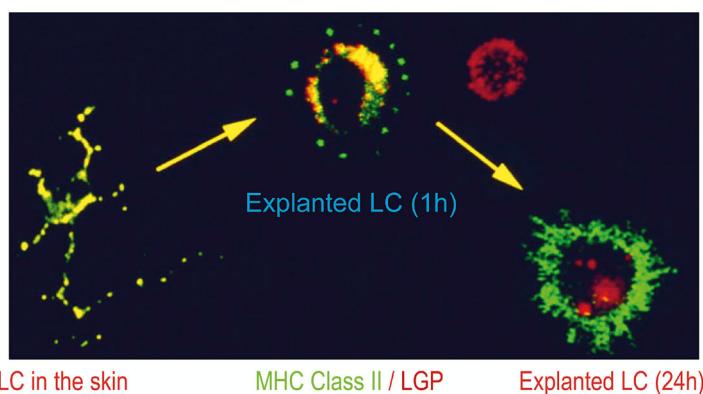


Figure 24. Cell biological basis for dendritic cell maturation. MHC Class II is found in vesicles inside the cell in the immature state and translocated to the cell surface in mature dendritic cells (Pierre et al., 1997). LGP is lysosomal membrane protein lgp-b/lamp-2. LC is Langerhans cell.

Based on these experiments, Steinman and his colleagues proposed the following model (Fig. 25). Dendritic cells can be found in two states of activation. In the immature state, they express antigen capture receptors and pattern recognition receptors that can induce activation. In the activated or mature state, they express high levels of co-stimulatory molecules for T cell activation and MHC surface antigens. The transition between these two states can be modulated by microbial products including Toll receptor ligands as well as by signals such as cytokines and ligation of the costimulatory protein CD40 (Fig. 25).

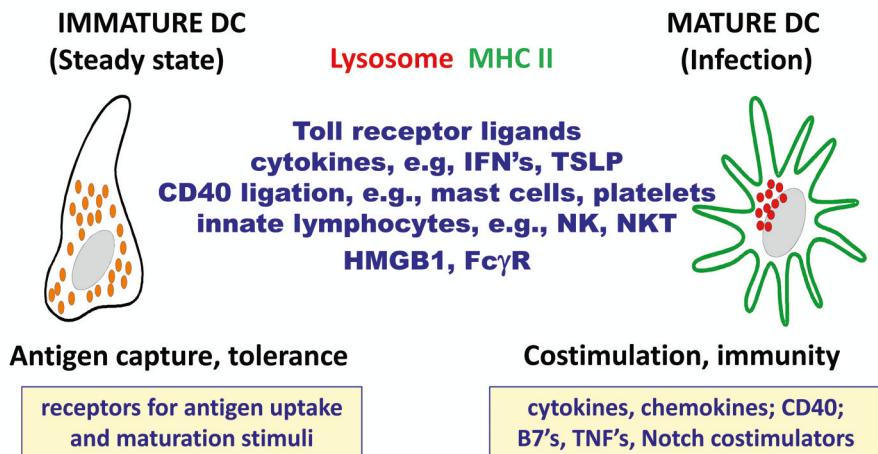


Figure 25. The diagram summarizes the idea that dendritic cells act as sensors which can be induced to undergo maturation as a result of Toll like receptor signaling or signaling by a number of other pathways. In the immature state dendritic cells are specialized for antigen capture, and in the mature state they up-regulate surface molecules required for T cell activation and polarization.

Steinman then suggested the following paradigm (Fig. 26). When a dendritic cell at a body surface receives an innate signal from the incoming pathogen by virtue of, for example, Toll receptor ligation, it becomes activated or mature. The mature dendritic cell then migrates to the local lymph node to join the networks of dendritic cells that contact migrating T and B cells. In addition to presenting antigen, the dendritic cell orchestrates the adaptive immune response by activating effector T cells. The activated T cells then leave the dendritic cell network in the lymphoid organ and patrol the body for invading pathogens.

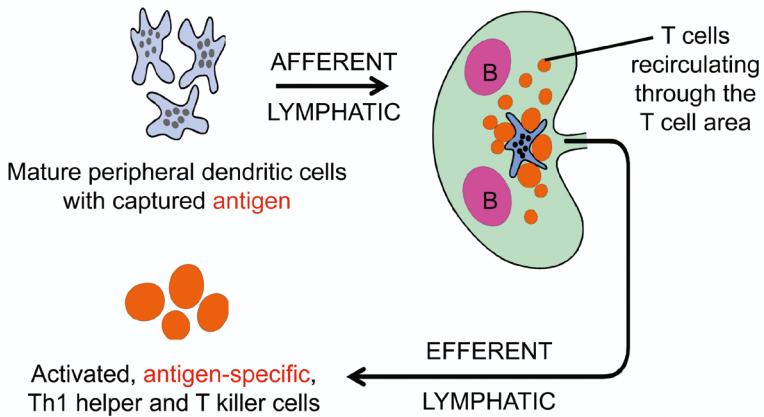


Figure 26. Proposed pathway for antigen capture and activation by peripheral dendritic cells and traffic to the lymph nodes where they present antigen to T cells. Activated T cells leave the lymph nodes and circulate in the organism.

IMMUNE TOLERANCE

An important test of the idea that dendritic cells initiate immunity was to deliver an antigen to dendritic cells *in vivo*. Until 2001 all experiments showing that dendritic cells activate immunity had been performed by removing dendritic cells from their natural environment and testing their function after *in vitro* manipulations. Daniel Hawiger, a graduate student in Nussenzweig's laboratory devised a system to test dendritic cell function *in vivo* by delivering antigen to these cells *in situ* (Hawiger et al., 2001). What he did was to engineer a monoclonal antibody specific to a molecule on dendritic cells, DEC-205 (Jiang et al., 1995) (Fig. 27), that would then serve as a specific delivery vehicle to carry the antigen to the dendritic cell (Fig. 28). When injected into a mouse, the chimeric antibody bound to dendritic cells and thereby delivered the antigen. Antigen delivered to dendritic cells in this manner was far more efficient than soluble antigen in inducing T cell responses (Fig. 29). Moreover, because antibodies have a long half-life (Fig. 30), their targeting of dendritic cells lasted for days, and there were prolonged T cell responses with immunity strong enough to reject tumors or handle a viral infection (Bonifaz et al., 2004)

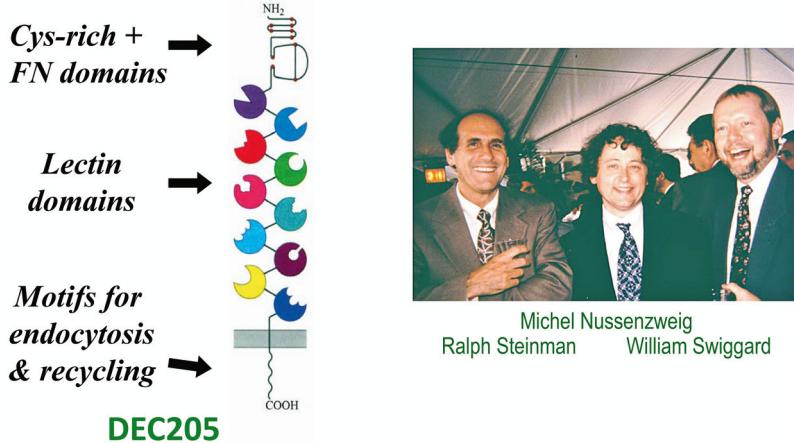


Figure 27. Diagram shows the domain structure of the DEC-205 molecule (left). Photograph of Ralph Steinman, Michel Nussenzweig and William Swiggard, who with Wanping Jiang cloned the molecule (Jiang et al., 1995).

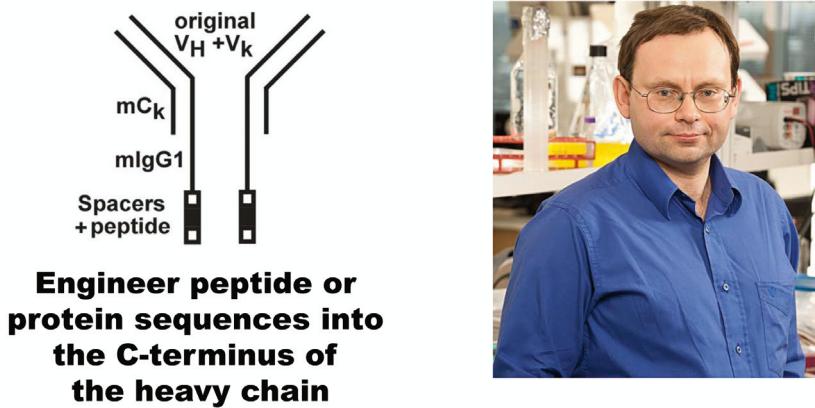


Figure 28. The diagram shows strategy for delivering antigens to dendritic cells in vivo using anti-DEC-205 fusion antibodies (left) developed by Daniel Hawiger (photograph right) (Hawiger et al., 2001).

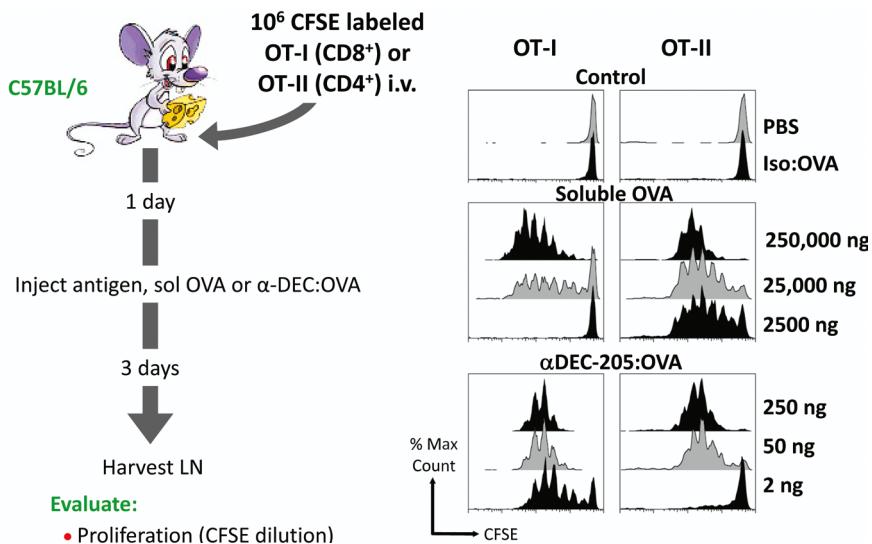


Figure 29. Dendritic cell targeted antigen is far more efficient than soluble antigen in inducing T cell responses. Experimental protocol (left) and OTI class I restricted, ovalbumin specific CD8+) or OTII (class II restricted, ovalbumin specific CD4+) T cell responses to injected soluble antigen, or antigen targeted to dendritic cells by DEC-205 (right) (Hawiger et al., 2001). OVA is ovalbumin.

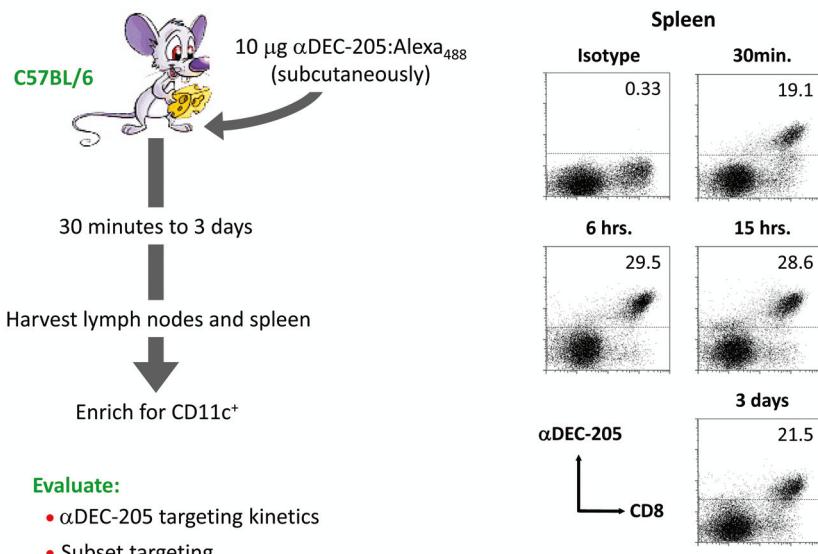


Figure 30. Dendritic cell targeting by DEC-205 fusion antibodies results in long lasting antigen targeting to dendritic cells. Experimental protocol (left) and time course showing long lasting dendritic cell associated DEC-205 in vivo (right) (Hawiger et al., 2001).

When dendritic cells were activated by Toll receptor ligation or some other stimulus by giving antigen along with a maturation stimulus, the result was robust immunity (Fig. 31, (Hawiger et al., 2001)). But when the antigen was administered alone, T cells, instead of becoming effector cells for immune responses, were stopped from responding by one of a number of different mechanisms. They were either deleted, silenced (anergized), or actively induced to become regulatory T cells (Fig. 32, (Hawiger et al., 2001; Hawiger et al., 2004; Kretschmer et al., 2005)).

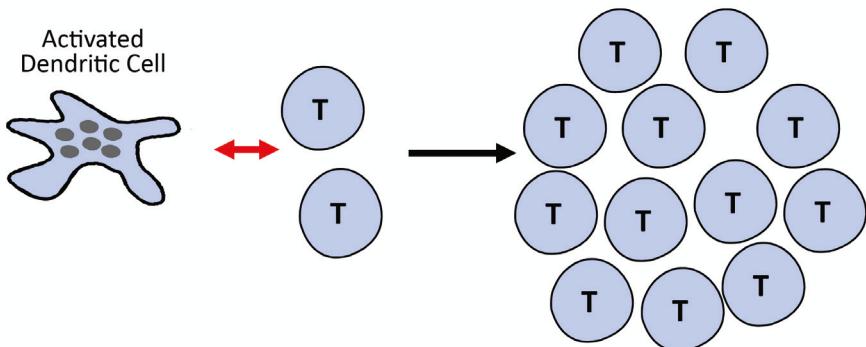


Figure 31. The diagram shows antigen specific T cell clonal expansion and activation by dendritic cells that present antigen after activation by CD40 or Toll receptor ligands (Hawiger et al., 2001).

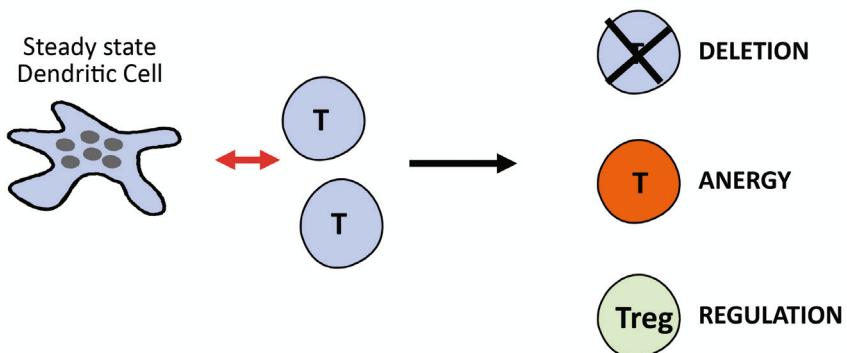
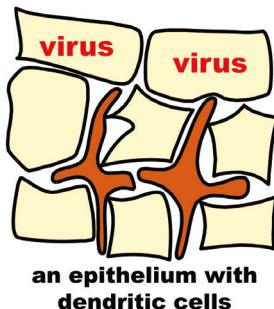


Figure 32. The diagram summarizes the observation that antigen presentation by steady state dendritic cells results in T cell tolerance by deletion, anergy or induction of T regulatory cells (Hawiger et al., 2001; Hawiger et al., 2004; Kretschmer et al., 2005).

This important experiment added an unanticipated role of dendritic cells to maintain tolerance. The model put forward by Nussenzweig and Steinman is that under resting conditions, dendritic cells are continually capturing self-antigens from serum or dying cells. These antigens are also continu-

ally being presented to T cells. In the absence of an activation signal, the self-reactive cells are silenced (Steinman and Nussenzweig, 2002). This is one of the mechanisms by which the immune system averts Ehrlich's horror autotoxicus. During viral infections, for example, the dendritic cells pick up a mixture of self and non-self antigens, all of which are presented to T cells. The previous silencing of the self-reactive cells allows the immune system to avoid attacking self and to focus on the invading viruses, bacteria, or toxins (Fig. 33).

Self and harmless environmental antigens



an epithelium with dendritic cells

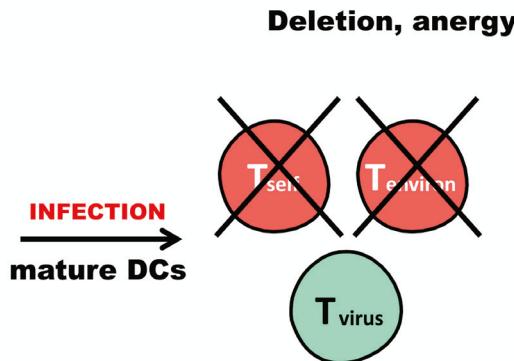


Figure 33. Induction of T cell tolerance by steady state DCs is required to prevent anti-self reactivity during immune responses to pathogens. Steady state dendritic cells capture, process and present self-antigen to T cells resulting in tolerance. The same self-antigens are also processed and presented by dendritic cells when they capture pathogen-infected cells, but the immune response is focused on the pathogen because the anti-self reactive T cells were previously silenced in the steady state (Steinman and Nussenzweig, 2002).

Thus, the dendritic cell discovery and its role in directing both tolerance and immunity not only connects innate and adaptive immune responses but also helps to explain how self-reactivity is removed from the adaptive repertoire to prevent autoimmunity, or Ehrlich's horror autotoxicus.

DENDRITIC CELL LINEAGE AND DEVELOPMENT

From the beginning, Steinman was interested in the relationship of dendritic cells to monocytes and macrophages. Many laboratories have contributed to solving this problem, but the first breakthrough came from Frederic Geissman (Fogg et al., 2006). He fractionated developing bone marrow cells looking for progenitors of dendritic cells and found that myeloid progenitors could give rise to dendritic cells whereas lymphoid progenitors did not. Most importantly, Geissman defined a bone marrow progenitor that was restricted to producing dendritic cells and monocytes, but not lymphoid cells or granulocytes (Fogg et al., 2006).

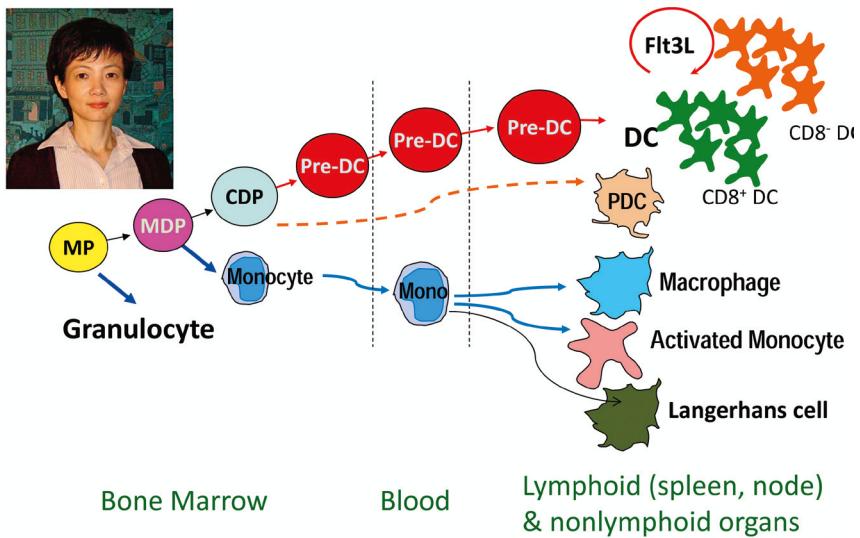


Figure 34. Dendritic cell development. Kang Liu (photograph left) and a diagram of the cellular intermediates in the dendritic cell development pathway in the bone marrow blood and tissues. The dendritic cell pathway splits from the monocyte pathway in the bone marrow after the common macrophage and dendritic cell precursor state. Pre-dendritic cells leave the bone marrow and enter the tissues (Liu et al., 2009). MP is myeloid progenitor, MDP is monocyte-dendritic cell progenitor, CDP is common dendritic cell progenitor, and PDC is plasmacytoid dendritic cell.

Kang Liu and Claudia Waskow accomplished the next key experiments during their postdoctoral fellowships in Nussenzweig's laboratory (Fig. 34). (Liu et al., 2009; Waskow et al., 2008) They found that monocyte and dendritic cell lineages split from each other in the bone marrow. The monocyte-dendritic cell progenitor discovered by Geissman produces monocytes and also produces a more developed progenitor that is committed to the dendritic cell lineage and can no longer produce monocytes. Instead, this dendritic cell committed progenitor is limited to producing plasmacytoid dendritic cells and pre-dendritic cells that leave the bone marrow and enter tissues where they further divide in response to a hematopoietic growth factor called Flt3L to give rise to the two major subsets of dendritic cells in lymphoid and non-lymphoid tissues. Exactly how these differentiation steps are controlled is still not known.

DENDRITIC CELL-BASED VACCINES

Steinman liked to emphasize that vaccines by and large have not been created by immunologists. Instead microbiologists, like Pasteur, used attenuated microbes to stimulate the immune system. Steinman's frequently stated goal was to use immunology to create vaccines. He spent the last four years of his life investigating ways to harness dendritic cells to produce vaccines.

The features of dendritic cells that Steinman wanted to exploit to produce immunity were 1) specific receptors for antigen uptake and processing,

such as DEC-205; 2) pattern recognition receptors that activate or mature dendritic cells, such as the Toll like receptor ligand; and 3) the various pathways of dendritic cell development into their different subsets (Fig. 35).

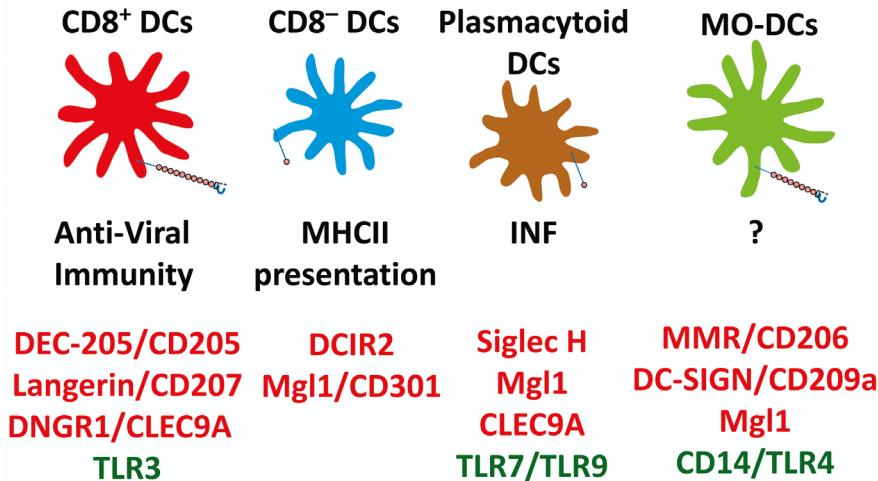
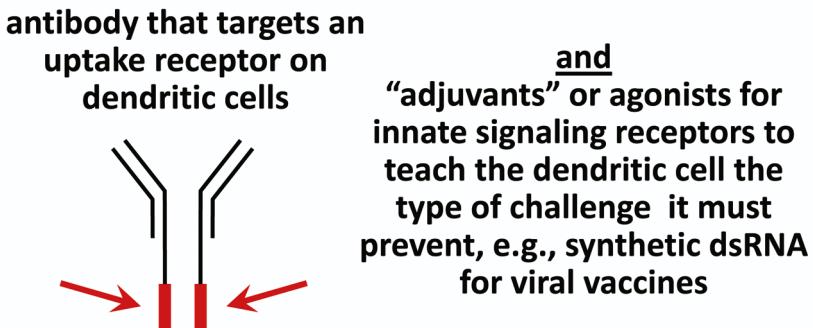


Figure 35. Subsets of dendritic cells, their functional specializations, and receptors that might be used to target or activate each one specifically.

The conceptual framework for Steinman's new vaccines was based on Daniel Hawiger's experiment (Hawiger et al., 2001), that is, using antigens specific for HIV, tuberculosis, allergy, diabetes, or cancer tied to a specific antibody serving as a delivery vehicle that would reach dendritic cells (Fig. 36). In addition, this type of immunization would require different adjuvants to activate dendritic cells. Unlike other vaccines, this one would be delivered to dendritic cells throughout the body because the antibody would carry the antigen to all dendritic cells. The idea was that by using different receptors to target different dendritic cells and different innate stimuli, these vaccines could activate different types of immunity. For example, Steinman ambitiously considered and planned vaccines to prevent or treat cancer and infection as mediated by Th1 and Th2 immunity as well as vaccines to regulate allergy and inflammation by inducing regulatory T cells.



Protective antigens for AIDS, cancer, autoimmunity (e.g., multiple sclerosis)

Figure 36. Suggested approach to dendritic cell-based vaccines for humans. Antibodies to endocytic receptors on dendritic cells are used as fusion proteins to target antigens to specific dendritic cells *in vivo* (left). Toll receptor ligands or other agents activate dendritic cells in specific ways (right).

Steinman’s success in mouse models with anti-cancer (Fig. 37) and anti-viral immune responses encouraged him to use this approach in humans. His clinical group at The Rockefeller University Hospital is currently conducting the first proof-of-concept study in a phase one clinical trial (Fig. 38). The vaccine consists of a HIV gag p24 protein engineered into anti-DEC-205 antibody and is administered together with a Toll receptor 3 ligand (Poly ICLC) as the adjuvant to mature the dendritic cells.

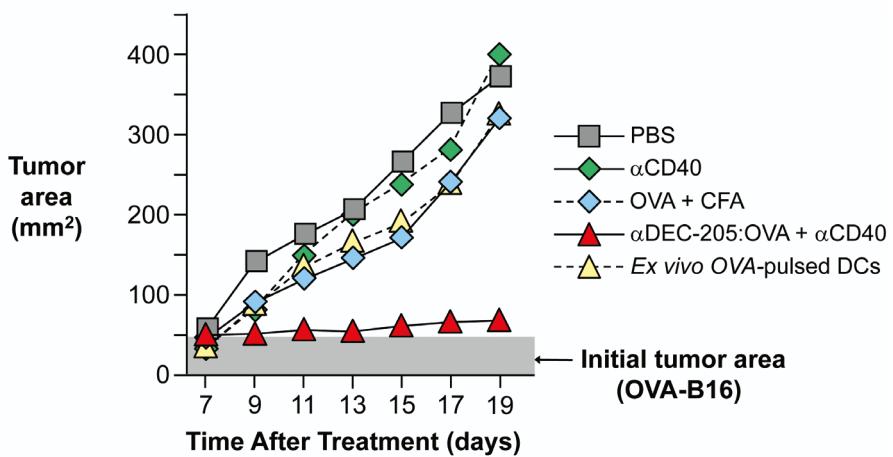


Figure 37. Strong protective immunity to B16-OVA melanoma after vaccination with anti-DEC-205-OVA and CD40 ligation. X axis is time after tumor challenge, Y axis is tumor size. OVA is ovalbumin. (Bonifaz et al., 2004).



Figure 38. Ralph Steinman's clinical team standing in front of The Rockefeller University Hospital. Top left to right: Ralph Steinman, Godwin Nchinda, Leonia Bozzacco, Christine Trumpheller, Sarah Schlesinger, Jules Hoffman. Bottom, left to right: Paula Longhi, Marina Caskey, Niroshana Anandasabapathy, Sarah Pollak, Irina Shimeliovich. 28 April 2009, courtesy of The Rockefeller University.

EPILOGUE

Ralph Steinman created a revolution in immunology when he discovered a beautiful cell by just looking through a microscope. During the following forty years at The Rockefeller University, he characterized this cell and elucidated its roles in immunity. He showed that dendritic cells are critical for initiating the most important immune responses. He described their three central features. Dendritic cells are “Sentinels” that capture pathogens, as Metchnikov suggested. They are “Sensors” for infection that use their cell surface pattern recognition receptors like Toll to become activated. And, once activated, they become “Conductors” of the immune orchestra whose individual cells play harmonious roles to protect and regulate the body’s immune system. Dendritic cells link Metchnikov to Ehrlich (Fig. 39).

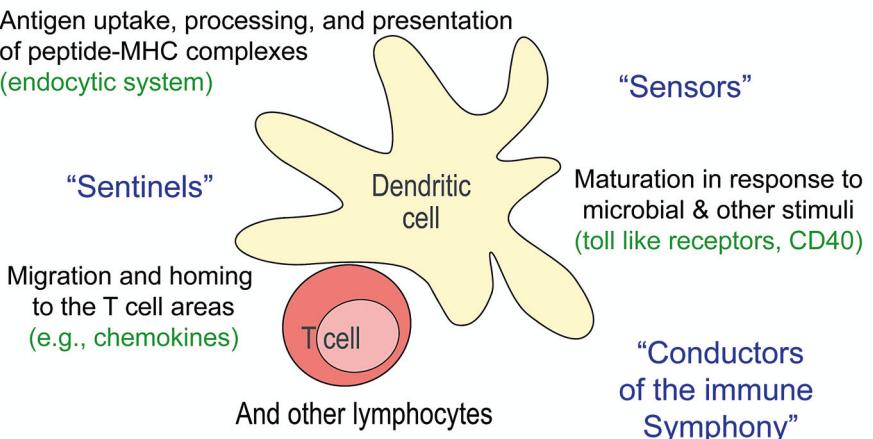


Figure 39. Dendritic cells are innate cells that activate adaptive responses. The diagram shows that dendritic cells are sentinels positioned in tissues to detect pathogens or inflammation; they act as sensors by virtue of expression of receptors that detect pathogens or other inflammatory signals; and they are conductors of the immune symphony as they process and present antigen to activate adaptive immune responses.

Ralph Steinman was excited in 1973 when he discovered dendritic cells. And in 2011, he was confident they would form the basis of still unknown medical advances.

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