NOVARTIS IMMUNOLOGY PRIZEWINNING LECTURE

Dendritic cells: from the fabric of immunology

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I am so grateful for this year's Novartis prize in basic immunology. This is a great honour and a deeply appreciated celebration of dendritic cells (DCs). I would like to recall the early days of the field and illustrate how DC biology arose from the methods, findings and concepts of many scientists who were studying DCs as well as other topics in immunology. We all realize that immunology is an interwoven fabric of many distinct and beautiful parts. This framework was critical for discovering DCs and some of their properties.

I have spent my life in the northeast of North America and received my education at Sherbrooke High School, McGill University and Harvard Medical School. During my medical training at Massachusetts General Hospital in 1968, I attended a series of lectures on the "new cellular immunology." The field was about to take off, armed with the incisive clonal selection theory and new knowledge that distinct B and T lymphocytes were the mediators of two kinds of immunity: humoral and cellular. These lectures made me decide that immunology was for me. It was full of unknowns in physiology that would benefit from cell biological approaches and rich in significance for medicine, in which so many diseases involve the immune system.

I managed to gain a postdoctoral position at The Rockefeller University in 1970 to work with Zanvil Cohn and James Hirsch, both physicians who were leaders in the study of phagocytes. Their laboratory was begun by René Dubos, who was then an emeritus professor. Dubos was a microbiologist focused on tuberculosis. He provided a broad perspective with his emphasis on the interaction of the host and its environment, and he had just won a Pulitzer Prize for nonfiction in 1969 for his book, *So Human an Animal*. All three were brilliant scientists and mentors, and their outlooks were preciously positive. All three were interested in cell-mediated immunity against infectious diseases and were advisors to the Trudeau Institute in Saranac Lake, New York, where these fields were being pioneered by George Mackaness, Douglas MacGregor and Robert North.

It was evident during medical training and clearly impressed upon me during my early contacts at the Trudeau that there were real mysteries to the initiation of immunity, including resistance to infection.[1] The skin test for tuberculosis is an example. Tuberculin antigen from *Mycobacterium tuberculosis* is *recognized* when injected into individuals who have been exposed to tuberculosis or have been vaccinated with BCG [bacillus of Calmette and Guérin]. However, naïve individuals like myself do not *respond* to this foreign antigen, even after several dozen annual skin tests. Another example of the mystery in initiating immunity would be the work of Peter Medawar,[2] the father of transplantation biology. He showed that an immune mechanism was responsible for graft rejection, and he demonstrated that specific tolerance could be established neonatally to foreign cells. Yet, he spent many years trying to isolate active transplantation antigens that could elicit immunity or mimic the potent immunogenicity of a living graft, but he could not do so. This problem, the requirements for responsiveness, has always motivated my research. Initially, my question was: what stands in the way of antigen and the initiation of immunity?

After I had done some research on the cell biology of endocytosis and the handling of proteins by macrophages,[3,4] I decided to study the available system for initiating antigen-specific immunity. In this system, developed in 1967 by Robert Mishell and Richard Dutton,[5] T-cell–dependent antibody responses to sheep red blood cells required both lymphocytes and so-called "accessory cells." I wondered whether these accessory cells would reveal some-

thing important about the initiation of immunity. This may seem like a black box today, but Mishell–Dutton cultures were pivotal for immunology in the 1970s for identifying critical cell–cell interactions and underlying mechanisms.

In 1973, Zan Cohn and I spotted unusual cells in the accessory populations of spleen, which we named dendritic cells after the Greek *dendreon* or tree.[6] Their tree-like processes continually formed and retracted in the living state. I tried hard to come up with a term that was easier to pronounce than *dendritic*, but failed. One term I considered was *claudiacyte*, because my wife Claudia has attractive long extremities. Actually, Claudia has for more than 30 years been an incomparable partner to me and parent to our children. She has made so many things possible, including a special interest in clonal expansion when we and our son Adam were blessed with genetically identical but otherwise unusually different twin daughters, Lesley and Alexis.

Let's return to the early identification of DCs and the criteria we used to identify them as a distinct cell lineage and to prepare enriched populations for further studies. The laboratory was a beehive of special scientists and friends, including Sam Silverstein, Carl Nathan, Jay Unkeless, Ira Mellman, William Muller, Ralph van Furth and Siamon Gordon. Each was studying the biology of macrophages with Zan and Jim, and Siamon had already begun his illustrious career on macrophage development and markers. I found that the accessory populations from spleen contained both macrophages and DCs, which differed in appearance and other markers.[6,7] After a fair amount of struggle, I learned how to enrich the DCs. One of the features I noted was that all DCs expressed MHC [major histocompatibility complex] class II products or "Ia" antigens, as they were then called.[8] Macrophages, in contrast, were often MHC class II low, although Ira Mellman and I found that one could induce the synthesis and expression of macrophage "Ia" with lymphokines.[9] We would not have turned to MHC II if it were not for the spectacular discovery of Ir genes by Hugh McDevitt and colleagues[10,11] and the development of antisera to Ia antigens by David Sachs, Günter Hammerling, and others.[12–14]

Because of the presence of Ia antigens on DCs, we set out to verify that they could stimulate the mixed leukocyte reaction (MLR). This reaction was discovered independently in the two places I call home, New York and Montreal.[15,16] The MLR had been in use for nearly 15 years to type prospective donors and recipients of transplants, as it detected genetic incompatibilities in MHC products. The evidence was that Ir genes, when expressed as Ia antigens on stimulator cells, initiated the MLR.[17–19]

However, when we tested different cells as stimulators, the DCs were powerful initiators of immunity, at least a hundred times more potent than bulk spleen cells.[20] In contrast, enriched Ia-positive B cells and macrophages were weak stimulators.[9,20] Quantitative dose–response curves and comparisons of DCs with other cell types were always carried out to show the distinct functions of DCs during our first decades of DC research. In 1978, I suggested that "DCs would prove to be a critical accessory cell required in the generation of many immune responses" and that "DCs would use other accessory functions, in addition to antigen presentation (here expression of transplantation antigens), to initiate immunity."[20]

My first two graduate students to study DCs, Wesley van Voorhis and Michel Nussenzweig, then solved critical problems. It was Wes who identified DCs in human blood, and he began to use cell sorting to study DC and monocyte function. He employed a FACS [fluorescence-activated cell sorter] II, the second commercial version of Leonard Herzenberg's brainchild.[21–23] Michel studied mouse DCs, quantifying their high levels of MHC class II products[24] and developing the first DC-restricted antibody, 33D1.[25] Michel also was the first to show that DCs could present nominal antigens using trinitrophenyl (TNP)-modified cells,[26] a system pioneered by Gene Shearer. The DCs presented irradiated TNP-modified T cells and initiated a cytolytic T-lymphocyte response. In the initial review on DCs in 1980, we concluded that "the new DC lineage should have major functions in the sensitization or afferent limb of the immune response and in the biology of the MHC."[27]

Other laboratories were extending the MLR data to DC function in authentic transplantation in rodents.[28–30] It may seem expedient, when receiving a prize from Novartis, to pay homage to transplantation immunology, but, in truth, transplantation has been a

fountain of youth for DC biology and, more broadly, for immunology.

Let's return to the lab and the arrival of Kayo Inaba in 1981. She was pivotal for many early discoveries on DCs. Prior to coming to New York, in her PhD work in Kyoto, Inaba had found that antibody responses required an accessory cell different from a typical macrophage.[31] She quickly confirmed that DCs were major and potent accessory cells for antibody formation in Mishell–Dutton cultures, including experiments in which Nussenzweig's 33D1 antibody was used to ablate the response.[32]

Inaba established that antigen-specific responses in the MLR and in Mishell–Dutton cultures developed in clusters of DCs and T cells. From these clusters, she could isolate antigen-specific T cells that had been activated by DCs. She established that DCs mediated the afferent limb of immunity, initiating the formation of MHC-restricted CD4+ helper T cells. The activated T cells then helped B cells, matched to the MHC of the DC, to grow and produce antibody in the efferent limb of immunity.[33,34] Inaba and James Young made comparable findings on two stages for CD8+ T-cell responses.[35,36] DCs initiated the growth and differentiation of CD8+ cytolytic T lymphocytes, and these killers acted on other targets presenting antigen.

Maggi Pack and I were at the same time examining the localization of antigen-presenting cells in vivo by staining tissue sections with monoclonal antibodies, a new technology at the time.[37] For 20 years, Pack has led our studies of DCs in tissue sections. As a graduate student, she also showed that DCs responded to granulocyte–macrophage colony-stimulating factor (GM-CSF).[38] We found that MHC class II rich DCs were abundant in the T-cell areas of peripheral lymphoid tissues, an ideal location to initiate T-cell immunity.

We also turned to Langerhans cells in the skin after I was joined by two Tyroleans, Gerold Schuler in 1984 followed by his colleague, Nikolaus Romani, in 1987. Their skills with Langerhans cells, acquired in Innsbruck, were impressive, much like the mountains of Tyrol. The work of Steve Katz and David Sachs had revealed that Langerhans cells were bone marrow–derived[39] and not neural or neural crest cells as once believed. Others, especially Georg Stingl and Ethan Shevach, had shown that Langerhans cells could present antigens.[40] Schuler and Romani asked whether Langerhans cells were related to DCs.

Surprisingly, when Schuler studied Langerhans cells in culture, the cells needed to differentiate or mature in many ways before acquiring the strong stimulating function of DCs for the MLR.[41] Romani took the maturation concept further and found that antigen capture selectively took place in the immature stage, whereas mature cells were potent stimulators of resting T cells.[42–44] The lack of antigen uptake by mature immunostimulatory DCs ("antigen presenting cells") was surprising, even shocking. We therefore suggested that both uptake and maturation were critical for initiating immunity.

Many investigators around the world were also identifying DCs in different sites, so that a consensus view emerged by the late 1980s in which DCs in peripheral tissues[45–52] and transplants[28–30] could capture antigens, mature, and move via the lymph[53–58] or blood[59] to the T-cell areas[60–64] to initiate immunity.

However, there still was relatively little direct work on DC function in vivo. Kayo Inaba then returned for periods in the laboratory in 1989. We had found that DCs could be identified by their high expression of the CD11c integrin,[65] so we could use the cell sorter to separate DCs from mice that had been given soluble protein antigen. The CD11c+ DCs were the major source of stimulatory, MHC peptide complexes for T cells.[66]

Inaba, with graduate student Josh Metlay, also carried out in vivo priming with DCs. At this time, much of the important research on antigen presentation was taking place in tissue culture and with T cells that had already been activated beforehand. Instead, we set out to study antigen presentation in vivo and with naïve T cells. We found that DCs could initiate immunity to different proteins without the need for other adjuvants. This is why we called DCs "nature's adjuvants."[67]

By 1992, Schuler and Inaba learned to grow DCs from proliferating progenitors.[68,69] GM-CSF, the cytokine discovered by Don Metcalf in Australia, was pivotal. Talented scientists in many countries also made incisive and independent discoveries on DC development.[70–74] These findings made DCs more accessible and stimulated a major increase in research.

I want to conclude by emphasizing the international effort that was required for DC biology to enter the fabric of immunology. My comments have recognized more than fifty individuals from around the world who contributed significantly to the early days. Since then, hundreds of laboratories have continued to reveal new roles for DCs in immunity and tolerance, to uncover cellular and molecular mechanisms and, above all, to interface DC biology with problems in medicine. Accordingly, international congresses in DC biology, which have been organized every two years since 1990 by leaders in the field, have been vital and will remain so, for example in Bruges this October and in Edinburgh in 2006. I feel that it is critical that these meetings, as is also the case for this 12th International Congress in Montréal, serve as a link between fundamental and clinical immunology, because medicine sets high standards for scientific unknowns and challenges that we must address.

Louis Pasteur wrote: "Dans les champs de l'observation, le hasard ne favorise que les esprits préparés," or, the chance of discovery favours a prepared mind. This is true in part. What I have tried to review is something else that seems less emphasized, which is that discovery in DC biology and elsewhere repeatedly arises from a prepared profession like ours, a profession that is historically endowed, richly primed and continually evolving. DCs are a pilot light for a kitchen of fantastic immunological chefs. We are so lucky to be part of this profession and to be together at gatherings such as this. Thank you again for celebrating DCs, and long live the vigour and international spirit of immunology.

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