JEM Journal of Experimental Medicine 125 years

VIEWPOINT

JEM 125th Anniversary

The discovery of dendritic cells

Carol L. Moberg

Introduction by Carol L. Moberg, adapted from Moberg (2018)

One of Ralph's most satisfying allegiances was as editor of the *Journal of Experimental Medicine* (1978–2011). He helped transform it from an all-Rockefeller editorial board and a mid-century focus on immunology into a modern world-class journal. The new outside editors were active investigators who held weekly meetings to discuss the merits of manuscripts and to judge the soundness of their critiques. As the journal neared the beginning of its second century, Ralph pressed for a return to its historical balance of papers focused on the physiological and pathological relevance of research.

Moreover, he campaigned to move the coverage of immunology beyond in vitro studies and animal models to research on human subjects. This was a significant departure from the journal's original mission to publish from all areas of scientific medicine except clinical research. In several editorials, Ralph argued that human studies would provide a more systemic understanding of the manifestations and characteristics of disease. As he was learning about HIV in his own laboratory, the therapeutic procedures and theories of its pathogenesis were already calling for a return to the clinical setting.

As editor of the *Journal of Experimental Medicine* and several other journals, Ralph was uncompromisingly thorough and efficient in reviewing manuscripts. Papers had to convey conceptual advances, compelling data, and broad findings. He believed that publishing is an evolving process, as one paper could not have all the answers, so for science to go forward there would always need to be follow-ups and new approaches to address the unanswered questions. Thus, he was notably generous in encouraging submissions by authors who could make a convincing argument for the significance of their work.

The discovery of dendritic cells: An excerpt from Ralph Steinman's Harvey Lecture, delivered January 14, 2010

The story of dendritic cells began in 1970 after I joined The Rockefeller University laboratory of Zanvil Cohn, the founder of modern macrophage biology.

During my medical training, I was struck by a theory that explains how the immune system impacts so many spheres of medicine, each complex in its own right. The theory, since proven accurate, stated that the system contains a vast repertoire of lymphocytes, trillions of cells. Each cell had antigen receptors of a single specificity, but within the repertoire there were a few cells specific for proteins or other antigens relevant to each medical condition. During infection, for example with influenza, rare cells specific for individual viral proteins would be selected to expand, forming clones of millions or more influenza-specific protective cells. This clonal selection theory was the brainchild of MacFarlane Burnet (Burnet, 1957).

In addition to the importance of the immune system in medicine, there was a second challenge to understanding immunity, especially clonal selection. An infection like tuberculosis induces immunity, but when one studies individual proteins, like those in the skin test for tuberculosis, the proteins do not trigger an immune response unless one has previously been infected with *Mycobacterium tuberculosis* or received a vaccine to try to prevent this disease. Therefore, in the 1960s there was a gap. Foreign proteins could not act alone to initiate Burnet's clonal selection. Why not?

Carol L. Moberg and Ralph Steinman, 1991. Photograph courtesy of Lawrence R. Moberg.

This gap was also evident for transplant rejection, which is the most vigorous and infallible of immune reactions. Peter Medawar was a pioneer in proving that transplant rejection had an immune basis. His first paper in wartime England was a study of a burn patient receiving transplants of skin from her brother (Gibson and Medawar, 1943). The patient rejected the grafted skin more quickly the second time that she was engrafted, and Medawar realized that this heightened or memory type of response implied an immune basis. Yet Medawar later said, "We are still generally ignorant of how a homograft reaction starts" (Brent and Medawar, 1967).

The Rockefeller University, New York, NY.

Carol L. Moberg: moberg@rockefeller.edu.

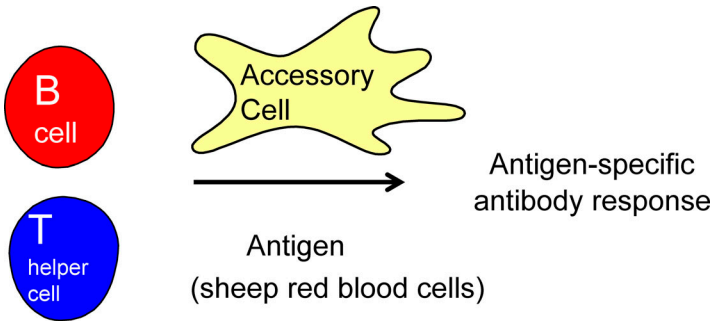

Figure 1. Cellular requirements for initiating antigen-specific immunity in mouse spleen cell cultures in 1970.

Medawar did not know why proteins isolated from grafts did not elicit immunity: they had to be presented by living dendritic cells to initiate rejection. Nor did I know this when I arrived at The Rockefeller University to study with Zan Cohn in 1970.

In my second postdoctoral year I decided to explore the spleen. Spleen cell suspensions were being used at the time to initiate immunity, or antigen-specific clonal selection, in what were called Mishell Dutton cultures (Mishell and Dutton, 1967). Surprisingly, in addition to lymphocytes and antigen, accessory cells needed to be added to the cultures (Fig. 1).

When we examined these heterogenous accessory cells, we saw something totally unanticipated. The population contained cells that did not look like any macrophage we had seen. We called the unusual cells dendritic cells (*dendreon*, Greek for tree), because in our cultures they continually formed and retracted processes or dendrites. James Hirsch, the coleader of the laboratory, helped me to watch and film the peculiar movements of living dendritic cells in vitro (Steinman and Cohn, 1973). Thirty years later, Michel Nussenzweig devised a transgenic mouse that allowed his student Randy Lindquist to observe these same continuous probing movements in the intact immune organs of mice (Lindquist et al., 2004).

Dendritic cells lacked all the known features of macrophages, including being poor at phagocytosis (Steinman and Cohn, 1974). Dendritic cells also lacked the abundant lysosomes detected cytochemically in macrophages, certain esterases, and the capacity to bind antibody-coated red cells (Steinman and Cohn, 1973). 33D1 was the first molecular marker for dendritic cells (Nussenzweig et al., 1982), along with high levels of MHC II or transplant antigens (Steinman et al., 1979). Using these distinctive features (Fig. 2), I was able to purify the dendritic cells and test their function.

Dendritic cells as potent initiators of immunity in tissue culture

Then there was another big surprise. Maggi Pack and I found that the enriched dendritic cells were powerful initiators of immunity, beginning with the initiation of transplant rejection in culture in the mixed leukocyte reaction (Steinman and Nussenzweig, 1980; Steinman and Witmer, 1978). Dendritic cells not only expressed high levels of transplantation antigens, they also had the capacity to use them to initiate immunity. Richard Batchelor and Robert Lechler in London, England then reported elegant experiments in which small numbers of dendritic cells, but not much large numbers of other cell types, induced graft rejection in rats (Lechler and Batchelor, 1982).

When we selectively removed dendritic cells from spleen with the 33D1 monoclonal antibody, much of the immune initiating activity was lost (Steinman et al., 1983). Likewise Denise Faustman with pancreatic islets (Faustman et al., 1984), and Kayo and Muneo Inaba with thyroid glands (Iwai et al., 1989), showed that 33D1-mediated depletion of dendritic cells from small endocrine organs allowed for their grafting across an MHC barrier. Thus, in the early 1980s, we felt we were on a valuable new track. Dendritic cells, distinct in morphology and other properties from macrophages and lymphocytes, also had a distinct, long sought function in the initiation of specific immunity (Nussenzweig et al., 1980).

Nussenzweig was the first student to work on dendritic cells, and he also came up with the first experiments to show that dendritic cells captured antigens and presented them to T cells (Nussenzweig et al., 1980). Another MD PhD student at the same time, Wes Van Voorhis, showed that dendritic cells could be identified in blood from humans (Van Voorhis et al., 1982). In parallel with Nussenzweig's 33D1 antibody, which killed dendritic cells but not monocytes, Van Voorhis was first to prepare a monoclonal antibody that did the opposite, killed monocytes but spared the active antigen presenting dendritic cells in the blood (Van Voorhis et al., 1983).

Kayo Inaba arrived in the laboratory in 1981. An elegant experimentalist, she discovered in her PhD work in Kyoto that macrophages were not responsible for accessory function in antibody responses in Mishell Dutton cultures (Inaba et al., 1981). We then showed that dendritic cells were responsible (Inaba et al., 1983). We also saw that the dendritic cells aggregated the T cells in the cultures, and found that these clusters were the microenvironment for generating immunity, e.g., nonclusters were depleted of

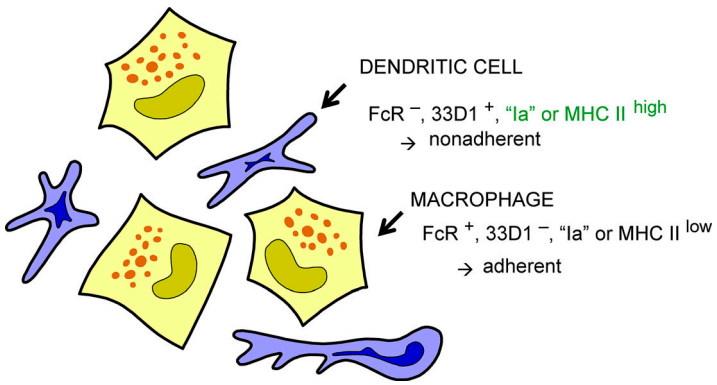

Figure 2. Some properties that were initially used, in addition to cytological differences, to distinguish macrophages and dendritic cells, and to purify them from mouse spleen.

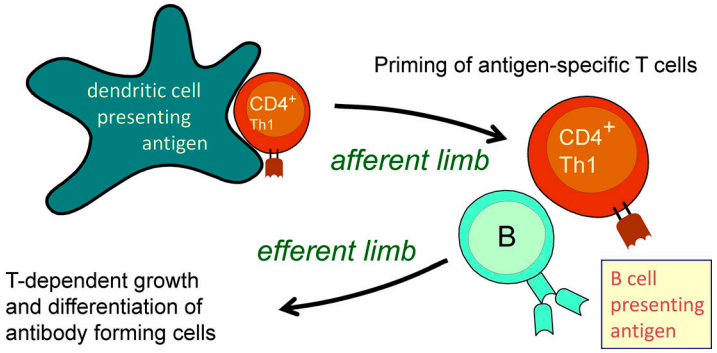

Figure 3. Two stages for antigen presentation during immunity, as illustrated by T helper–dependent antibody production. In the initial afferent limb, sentinel dendritic cells select T cell clones specific for presented antigens and initiate T cell growth and differentiation. In the subsequent efferent limb, the activated T cells recognize antigens presented on other effector cells, such as antibody-forming B cells shown here.

antigen reactive clones (Inaba and Steinman, 1984; Inaba and Steinman, 1985). The clusters were beautiful to watch in the living state or by scanning EM, since they were covered with probing, dendritic cell processes. Pack also identified dendritic cells in the T cell areas of lymphoid tissues (Witmer and Steinman, 1984). Nussenzweig's (Shakhar et al., 2005) and other laboratories have watched these dendritic cells select clones of T cells in living immune organs. Inaba and Jon Austyn observed that, once activated, T cells can respond to antigens presented on the antibody forming B cells, leading to B cell expansion and antibody production (Austyn et al., 1983; Inaba and Steinman, 1985). James Young observed similar events with $CD8^+$ killer T cells, i.e., dendritic cells first activate specific $CD8^+$ T cells in clusters, and then the T cells leave the clusters to kill their targets (Young and Steinman, 1990). The late Sumi Koide found that T cells activated by dendritic cells could also trigger macrophages to make interleukin-1 in an antigen-dependent and MHC-restricted manner (Koide and Steinman, 1987). Thus it became apparent that immune responses were comprised of an afferent limb, where dendritic cells initiated immunity, and an efferent limb, where T cells and other antigen presenting cells propagated immunity (Fig. 3).

Dendritic cells as initiators of immunity in mice and men

We then moved to in vivo research. Inaba along with Josh Metlay and Koide isolated dendritic cells directly from mice (Inaba et al., 1990). About the same time, Inaba and Gerold Schuler developed a tissue culture system to grow dendritic cells from progenitors (Inaba et al., 1993; Inaba et al., 1992a; Inaba et al., 1992b). The dendritic cells obtained by either of these approaches were then charged with foreign proteins or bacteria and reinjected into the animals. The cells elicited immunity that was restricted to the MHC products of the injected dendritic cells. We therefore called dendritic cells "nature's adjuvants," because they could enhance immunity in the intact animal (Steinman, 1991). In sum, the barrier between proteins and immunity was beginning to fall. One needed to deliver antigens on dendritic cells.

Maturation energizes antigen presenting dendritic cells to initiate immunity

We encountered another big surprise when first Schuler and then Nikolaus Romani from Innsbruck, Austria came to do research in the laboratory: dendritic cells not only had to capture antigens, they also had to differentiate irreversibly or mature (Schuler and Steinman, 1985; Romani et al., 1989). This was a departure from the thinking at the time, which solely emphasized antigen uptake and processing in the initiation of immunity.

Maturation entails many changes. For example, expression of many cell surface molecules were either up or down-regulated, including increased expression of the B7 family of costimulatory molecules like CD80/86 (Inaba et al., 1994). Ira Mellman and colleagues showed that most of the MHC molecules were in lysosomes of immature dendritic cells, but during maturation with bacterial lipopolysaccharide and other stimuli, antigenic peptides became complexed with MHC products in the endocytic system and moved to the cell surface, where they could select clones of T cells (Pierre et al., 1997; Inaba et al., 2000; Turley et al., 2000). The up-regulation of MHC products on the cell surface occurred with different types of dendritic cells and maturation stimuli. Other laboratories found that maturation included the production of cytokines and chemokines as well as the expression of chemokine receptors and integrins that drive dendritic cell homing to lymphoid tissues and binding of specific T cell clones. While dendritic cell maturation is the link to different types of immunity, pathogens also take advantage of this process. Sarah Schlesinger found that the Dengue virus infects immature dendritic cells via a lectin DC-SIGN (Tassaneetrithep et al., 2003). Paul Cameron and Melissa Pope (now Robbiani) showed how mature dendritic cells from blood and skin initiated intense infection with HIV when the dendritic cells interacted with cultured T cells (Cameron et al., 1992; Pope et al., 1994).

To summarize this historical review, a pathway was found to explain the initiation of immunity to specific proteins and transplantation antigens (Banchereau and Steinman, 1998). Antigens were presented by maturing dendritic cells, which often homed to the T cell areas of lymphoid organs to select specific clones from the repertoire envisioned by Burnet. Once activated, T cells exited the lymphoid tissue to find antigens presented at sites of infection, grafting, allergy or tumors—wherever disease is in progress.

References

Austyn, J.M., et al. 1983. *J. Exp. Med.* https://doi.org/10.1084/jem.157.4.1101
Banchereau, J., and R.M. Steinman. 1998. *Nature.* https://doi.org/10.1038/32588
Brent, L., and P.B. Medawar. 1967. *Br. Med. Bull.* https://doi.org/10.1093/oxfordjournals.bmb.a070517
Burnet, F.M. 1957. *Aust. J. Sci.* 20:67–69.
Cameron, P.U., et al. 1992. *Science.* https://doi.org/10.1126/science.1352913
Faustman, D.L., et al. 1984. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.81.12.3864
Gibson, T., and P.B. Medawar. 1943. *J. Anat.* 77: 299–310.
Inaba, K., et al. 2000. *J. Exp. Med.* https://doi.org/10.1084/jem.191.6.927
Inaba, K., and R.M. Steinman. 1984. *J. Exp. Med.* https://doi.org/10.1084/jem.160.6.1717
Inaba, K., and R.M. Steinman. 1985. *Science.* https://doi.org/10.1126/science.3160115
Inaba, K., et al. 1981. *J. Immunol.* 127:452–461.
Inaba, K., et al. 1983. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.80.19.6041
Inaba, K., et al. 1990. *J. Exp. Med.* https://doi.org/10.1084/jem.172.2.631
Inaba, K., et al. 1992a. *J. Exp. Med.* https://doi.org/10.1084/jem.176.6.1693
Inaba, K., et al. 1992b. *J. Exp. Med.* https://doi.org/10.1084/jem.175.5.1157

Inaba, K., et al. 1993. *J. Exp. Med.* https://doi.org/10.1084/jem.178.2.479
Inaba, K., et al. 1994. *J. Exp. Med.* https://doi.org/10.1084/jem.180.5.1849
Iwai, H., et al. 1989. *Transplantation.* https://doi.org/10.1097/00007890-198901000-00011
Koide, S., and R.M. Steinman. 1987. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.84.11.3802
Lechler, R.I., and J.R. Batchelor. 1982. *J. Exp. Med.* https://doi.org/10.1084/jem.155.1.31
Lindquist, R.L., et al. 2004. *Nat. Immunol.* https://doi.org/10.1038/ni1139
Mishell, R.I., and R.W. Dutton. 1967. *J. Exp. Med.* https://doi.org/10.1084/jem.126.3.423
Moberg, C.L. 2018. National Academy of Sciences. http://www.nasonline.org/publications/biographical-memoirs/memoir-pdfs/steinman-ralph.pdf
Nussenzweig, M.C., et al. 1980. *J. Exp. Med.* https://doi.org/10.1084/jem.152.4.1070
Nussenzweig, M.C., et al. 1982. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.79.1.161
Pierre, P., et al. 1997. *Nature.* https://doi.org/10.1038/42039
Pope, M., et al. 1994. *Cell.* https://doi.org/10.1016/0092-8674(94)90418-9
Romani, N., et al. 1989. *J. Exp. Med.* https://doi.org/10.1084/jem.169.3.1169
Schuler, G., and R.M. Steinman. 1985. *J. Exp. Med.* https://doi.org/10.1084/jem.161.3.526
Shakhar, G., et al. 2005. *Nat. Immunol.* https://doi.org/10.1038/ni1210
Steinman, R.M. 1991. *Annu. Rev. Immunol.* https://doi.org/10.1146/annurev.iy.09.040191.001415
Steinman, R.M., and Z.A. Cohn. 1973. *J. Exp. Med.* https://doi.org/10.1084/jem.137.5.1142
Steinman, R.M., and Z.A. Cohn. 1974. *J. Exp. Med.* https://doi.org/10.1084/jem.139.2.380
Steinman, R.M., and M.C. Nussenzweig. 1980. *Immunol. Rev.* https://doi.org/10.1111/j.1600-065X.1980.tb01042.x
Steinman, R.M., and M.D. Witmer. 1978. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.75.10.5132
Steinman, R.M., et al. 1979. *J. Exp. Med.* https://doi.org/10.1084/jem.149.1.1
Steinman, R.M., et al. 1983. *J. Exp. Med.* https://doi.org/10.1084/jem.157.2.613
Tassaneetrithep, B., et al. 2003. *J. Exp. Med.* https://doi.org/10.1084/jem.20021840
Turley, S.J., et al. 2000. *Science.* https://doi.org/10.1126/science.288.5465.522
Van Voorhis, W.C., et al. 1982. *J. Exp. Med.* https://doi.org/10.1084/jem.155.4.1172
Van Voorhis, W.C., et al. 1983. *J. Exp. Med.* https://doi.org/10.1084/jem.158.1.174
Witmer, M.D., and R.M. Steinman. 1984. *Am. J. Anat.* https://doi.org/10.1002/aja.1001700318
Young, J.W., and R.M. Steinman. 1990. *J. Exp. Med.* https://doi.org/10.1084/jem.171.4.1315