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INSIGHTS

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Dendritic cells: The first step

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Ralph M. Steinman's work on dendritic cells began in 1973 when he described and named the cells. Reminiscent of the late Justice Ginsburg's perspective that enduring change happens not suddenly but one step at a time, the paper (1973. *J. Exp. Med.* https://doi.org/10.1084/jem.137.5.1142) was notably the first step in many steps of important work that revealed the nature of dendritic cells.

When Ralph M. Steinman was awarded a share of the 2011 Nobel Prize in Medicine or Physiology for his discovery of dendritic cells, highlighted among his key discoveries was his first paper in 1973 characterizing and naming dendritic cells (Steinman and Cohn, 1973). It may surprise the first-time reader of this seminal paper that the work did not include a functional analysis revealing the role of dendritic cells in fostering T cell–mediated immunity. That would come later. Instead, the paper served as a basic stepping-stone from which Steinman, following his curiosity, would publish a series of papers over decades to come. These papers as a group would reveal, step by step, the central role of dendritic cells as key antigen-presenting cells, a body of work that unveiled itself simultaneously with the discovery of the nature of antigen presentation itself by others. That is, the 1973 paper emerged during a period of rapid growth in immunology that included the identification of MHC restriction in 1974 (Zinkernagel and Doherty, 1974) followed by an understanding that degraded, earlier phagocytosed antigens were salvaged as peptides to be presented on MHC molecules as the major means by which antigen presentation occurred (Allen and Unanue, 1984; Babbitt et al., 1985). So, it would have been nearly impossible for a full understanding of the nature of the dendritic cell to be clear at the onset. The accurate but rather demure nature of the paper points to a time when solid science was seen worthy of publication even if the full picture or "mechanism" was not known. I believe many would agree that the series of papers starting with the seminal paper in 1973, while each would likely be declared incomplete by present-day reviewers, was better published in the increments it was than waiting for the more complete compendium that would have emerged some years later. As one who was lucky enough to be mentored by Steinman in the late 1990s, I am reminded of his own frequent comment while training me to review papers that "the literature needs to move forward," by which he meant that not all open questions raised by a study have to be solved at once. This statement very much applied to his own 1973 paper.

Dr. Randolph with Drs. Jacques Banchereau and Ralph Steinman, 1998, at the Fifth International Symposium on Dendritic Cells in Pittsburgh, PA.

In the 1970s, Steinman and his mentor Zanvil Cohn were part of a prominent group

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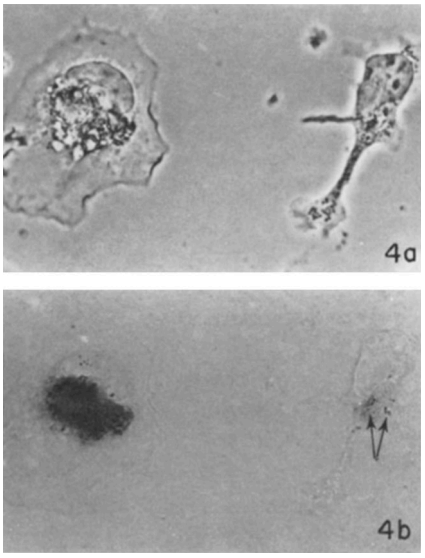

A panel from the work of Steinman and Cohn (1973) is reproduced here, with phase-contrast (a) and bright-field (b) imaging of a macrophage on the left and a dendritic cell on the right. More than a difference in cell shape and ruffling, the staining for acid phosphatase highlighted the lysosomes, which were clearly more numerous in macrophages and positioned in a perinuclear location in dendritic cells. Arrows point out small reactive granules.

at The Rockefeller University working on the nature of phagocytes and phagocytosis. The group included James Hirsch, who made major contributions to the early understanding of the cellular and molecular basis of phagocytosis. It is in that spirit that Steinman developed a project that was likely initially aimed at characterizing the macrophages of the mouse spleen. Steinman used basic techniques to characterize the cells of the spleen that adhered to glass coverslips: descriptive morphology and electron microscopic analysis of distinct patterns of organelles within the cells of differing morphology, and cinematography that documented behavior of cultured living cells that was truly state-of-the-art at the time and that allowed him to distinguish the ruffling behavior of macrophages from the cells he would name dendritic cells. The major conclusion of the work was that there was an arbor-shaped cell type that comprised 1% of all spleen cells. These cells, he argued, quite remarkably so considering the techniques available, were likely exclusively localized to splenic white pulp and were also present in lymph nodes and Peyer's patches. He noted that the newly christened dendritic cell had far fewer lysosomes than macrophages, and the lysosomes it had were nested next to the nucleus (see figure). Some two decades later, these distinct lysosomes would once again be described, with the conclusions extended to show that the perinuclear lysosomes could retain engulfed antigens for over 1 d, awaiting a signal for dendritic cell maturation that would rapidly relocate the sequestered antigen, displayed on MHC II molecules, to the cell surface for presentation to T cells (Pierre et al., 1997). In 1978, five years after his initial publication on dendritic cells, Steinman would publish that dendritic cells were rich in MHC molecules and had the capacity to drive T cell expansion in a mixed lymphocyte reaction (Steinman and Witmer, 1978). Some years later, the late G. Jeanette Thorbecke shared with me that she had suggested to Steinman to examine MHC II expression on his "lineage-negative cells," as she pointed out that he was fond of calling them at the time. Thorbecke and Ina Silberberg-Sinakin's work on Langerhans cells of the skin (Silberberg-Sinakin et al., 1976) would no doubt aid Steinman in later years in understanding the biology of dendritic cells in different stages of their life cycle and the important relationship between Langerhans cells and dendritic cells.

While Steinman and Cohn were convinced that dendritic cells were likely distinct from macrophages, they went on to support the argument further in future work, focusing on functional analyses that typified macrophages (Steinman and Cohn, 1974). In the 1973 paper, they conceded that they were uncertain whether the cells might nonetheless be what we now refer to as follicular dendritic cells. Then, as one of the next key steps, they worked out that the antigen-retaining reticular cells of the germinal center were not the same as their "lymphoid" dendritic cells (Steinman and Cohn, 1974).

As the follow-up steps to the original description of dendritic cells led Steinman to link dendritic cells to a remarkably potent capacity to stimulate T cells responses in a mixed lymphocyte reaction, Steinman became concerned with highlighting the role of dendritic cells as stimulators of immunity over that of macrophages (Inaba et al., 1983; Steinman et al., 1983). This point of concern was most likely driven by the fact that the elegant delineation of how antigen presentation occurred at the cell biological and molecular level, via the display of peptides derived from antigens degraded in the lysosomal compartment on major histocompatibility molecules, was worked out by Emil Unanue and colleagues in experiments that often used macrophages (Allen and Unanue, 1984; Unanue, 1984). However, in the literature, Unanue was careful to acknowledge that B cells and dendritic cells were also authentic antigen-presenting cells (Unanue, 1984), and indeed he highlighted the existence of thymic dendritic cells as potent antigen-presenting cells, suggesting that thymic dendritic cells should not be discounted as such simply because they displayed some differing properties to splenic dendritic cells (Beller and Unanue, 1980). This was a direct point related to the fact that Steinman and Cohn had noticed thymic cells resembling dendritic cells in their 1973 paper but failed to call them dendritic cells at the time (Steinman and Cohn, 1973), because they were not similar in every feature to those from the spleen.

Perhaps it was the argument that the superior phagocytic and degradative capacity of macrophages might position them best as antigen-presenting cells (Unanue, 1984) that drove Steinman to largely avoid the macrophage field. The quite-positive impact of this avoidance was that doing so gave space for the dendritic cell field to grow its distinct identity. And grow it did, through dedicated research and dendritic cell–focused conferences. These activities fed rapid expansion in understanding the developmental lineage of dendritic cells, a lineage that emerged clearly distinct from macrophages (Anderson et al., 2021). There was also strong growth in understanding the cell biology of dendritic cells that links to their potent antigen-presenting capacity, while certain areas of study, like cross-presentation, remain active frontiers with many unanswered questions (Theisen and Murphy, 2017). The negative impact of this avoidance was the fostering of a perception quite prominent in the 1990s and into the 2000s that dendritic cell and macrophage biologists were at odds and should not interact, and a few colorful personalities worked to deepen this divide (Hume, 2008). The naming of the 2015 Keystone meeting "Macrophages and Dendritic Cells Reunited" highlighted the historical separation of the subjects. Now, as a new decade is upon us, these barriers have largely been forgotten.

We can now appreciate that, during much of the 1970s and extending into the 1980s, the number of unanswered questions about antigen-presenting cells and the presentation of antigen itself were so numerous that it is remarkable how rapidly, accurately, and beautifully the scientific truth emerged from multiple angles at once. As so often is the case, science that some may have considered to be conflicting was, in fact, not at odds at all. By now, we accept not only the key role of dendritic cells in programming T cell–mediated immunity, but we further recognize the existence of distinct subsets of dendritic cells with unique functions. And, of course, there are common features in the processing and presentation of antigen by macrophages and dendritic cells.

Before the end of his life, Ralph Steinman dreamed of a time when dendritic cells would be targets of therapeutics to alter immune outcomes in diseases ranging from HIV to cancer. Indeed, although ongoing approaches are different than what he imagined, the promise of harnessing dendritic cells to control cancer is a reality in the making. And it may be the case that such therapies will go hand in hand with different therapies that also, and simultaneously, target macrophages. We are grateful for Ralph Steinman's curiosity that led him to follow a morphological observation with rigor and tireless passion for decades beyond the first study, a curiosity that resulted in accelerating our understanding of the complexity of antigen-presenting cells in modern immunology and how we might manipulate them for the good of health.

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