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Breakthroughs in Immunology

Dendritic cells: Understanding immunogenicity

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The impetus for the discovery of dendritic cells in 1972 was to understand immunogenicity, the capacity of an antigenic substance to provoke immunity. During experiments to characterize "accessory" cells that enhanced immunity, we spotted unusual stellate cells in mouse spleen. They had a distinct capacity to form and retract processes or dendrites and were named dendritic cells (DC). DC proved to be different from other cell types and to be peculiarly immunogenic when loaded with antigens. When Langerhans cells were studied, immunogenicity was found to involve two steps: antigen presentation by immature DC and maturation to elicit immunity. Antigen-bearing DC were also immunogenic *in vivo* and were therefore termed "nature's adjuvants". Several labs then learned to generate large numbers of DC from progenitors, which accelerated DC research. Tolerogenicity *via* DC, including the control of foxp3^{+} suppressor T cells, was recently discovered. Two areas of current research that I find intriguing are to identify mechanisms for antigen uptake and processing, and for the control of different types of immunity and tolerance. These subjects should be studied *in vivo* with clinically relevant antigens, so that the activities of DC can be better integrated into the prevention and treatment of disease in patients.

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Introduction

The driving force for the first sighting of dendritic cells (DC) in 1972 was to understand immunogenicity, which a pioneer in this field, Michael Sela, defined as "the capacity of an antigen to provoke an immune response" [1]. Sir MacFarlane Burnet had provided immunology with the critical clonal selection theory of immunity. It proposed that the immune system was comprised of a vast repertoire of clones, each expressing a distinct receptor to recognize an antigen. However, what stood between the administration of antigen and successful clonal selection, or immunogenicity? This remains a central theme in biology and medicine.

Sela, together with John Humphrey and Hugh McDevitt, began to unravel one critical component of immunogenicity. They had observed marked differences between strains of rabbits and mice in antibody responses to synthetic proteins that Sela had prepared [2]. McDevitt then proved that immunogenicity required "Immune Response" genes, which mapped to the MHC, now MHC II [3]. MHC-restricted presentation of viral antigens was then discovered by Peter Doherty and Rolf Zinkernagel for MHC I. Alain Townsend, Emil Unanue, Pam Bjorkman, Jack Strominger, and Don Wiley demonstrated that this was due to the ability of MHC I and II products to present peptide fragments from antigens, where the peptide-MHC complexes acted as ligands for the T cell antigen receptor. This seminal body of research identified a key component to immunogenicity, which is that proteins need to be *processed* and *presented* on MHC products before they can be recognized by T cells.

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Abbreviation: LC: Langerhans cells

Discovering dendritic cells

The antigen processing and presentation arm of immunogenicity was not known when DC were discovered in 1972. Instead, there was evidence that antigens were retained as *intact* proteins on the surfaces

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of what were called dendritic macrophages in the germinal centers of lymphoid organs [4, 5] (now termed "follicular dendritic cells") or on macrophages from the peritoneal cavity [6]. Together with my mentor Zanvil Cohn, a leader in macrophage cell physiology, we fed macrophages horseradish peroxidase, but we were unable to identify persistent intact antigen on the cell surface [7, 8]. Instead, the macrophage internalized HRP continually over long periods, and the bulk of the antigen was catabolized down to the level of amino acids. During these studies, we discovered that endocytosis required the rapid recycling of the internalized vesicle membrane [9, 10]. As revealed by Brown and Goldstein [11], the recycling of endocytic receptors allows all cells to continually capture adsorbed proteins for efficient degradation in lysosomes.

In the absence of persistent protein on the cell surface, we turned to lymphoid organs and in particular to mouse spleen, because these are the sites for the generation of immunity. We had no hypothesis about what we would find. Robert Mishell and Richard Dutton [12] had figured out how to generate primary antibody responses to sheep red blood cells using spleen cell suspensions. However, clonal selection could not take place, *i.e*. antibody responses would not develop, unless one added radioresistant *accessory* cells, along with the antigen, to mixtures of B and T lymphocytes. When I examined the populations of accessory cells, there was a Eureka moment. The accessory populations included unusual cells that did not look like any other white blood cell that had been seen before. The cells had a prominent stellate shape, and more impressively in the living state, continually extended and retracted their processes or dendrites. No other leukocyte moved in this manner. The cells lacked other key features of macrophages and lymphocytes [13, 14]. The novel cells were termed dendritic cells for "treelike," from the Greek "dendron" or "dendreon" for tree (Fig. 1).

A helpful choice, which took years to materialize, was to first enrich DC as a distinct cell type *prior* to studying their functional properties and comparing these to other cell types [15]. Others were using the expression of McDevitt's immune response gene-associated (Ia) antigens as a marker for functional accessory cells, but Ia antigens did not mark a specific cell type and later proved to be the MHC II products needed to present peptides. Instead, we and others used the distinct morphology of DC to identify, enrich and begin to characterize these cells in several tissues and species, and to prepare the first monoclonals, *i.e*. 33D1, now anti-DCIR2 [16–18] and N418 anti-CD11c [19, 20]. The distinct morphology that we used to guide our preparation of DC has now been observed in living lymph nodes, where each DC continually probes its environment by extending and retracting processes

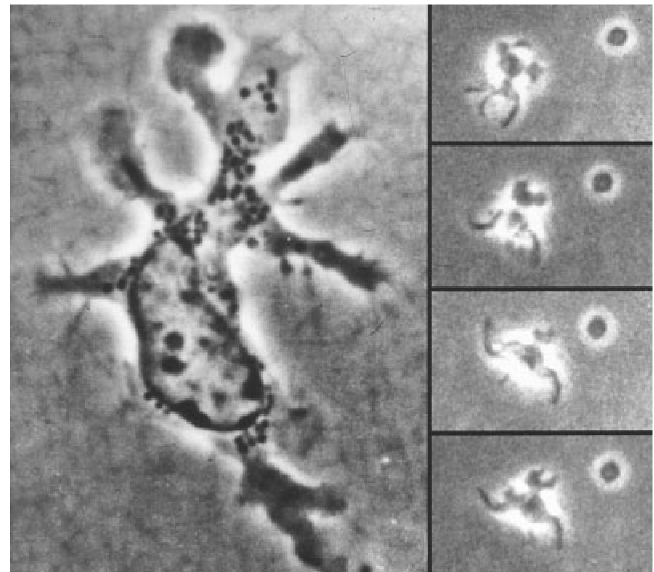

Figure 1. Phase contrast micrographs of dendritic cells. Left. A mouse spleen DC cultivated 24h in a plasma clot. Right. Four successive views of a living human blood DC cultivated in liquid medium to show the rapidly changing cell shape. The figure is reproduced with permission from [14].

[21]. The enriched DC also proved to have some surprising functions.

Dendritic cells as immunogens for transplantation reactions

The immunogenic role of DC was initially noted in the setting of transplantation, in a tissue culture model for graft rejection called the mixed leukocyte reaction (MLR) [22]. I like to recall that the MLR was discovered in the two places I call home, in Montreal by Barbara Bain *et al.* [23] and in New York by Fritz Bach and Kurt Hirschhorn, in 1964.[24] The MLR detects genetic incompatibility in the MHC between prospective donors and recipients of organ transplants. Incompatibility, which is frequent because of the striking polymorphism of the MHC, reliably leads to a T cell response that is typical of graft rejection. When Maggi Pack and I added graded doses of "donor" DC to stimulate rejection by "recipient" T cells, another exciting observation was made. The DC were at least 100 times more potent than total spleen cells, which contained about 1% DC [22]. Don Mason and Wesley Van Voohris [25, 26] made similar observations with DC from rat afferent lymph and human blood. We also noted that MHC II^{+} B cells and macrophages surprisingly were poorly immunogenic, although we later found that they were efficient in recalling a secondary MLR from T cells that had already been activated by DC [27]. In addition, we could directly observe the clustering of DC with the T cells that were

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generating immunity, thereby directly defining a DC microenvironment for generating immunity [28, 29]. The existence of DC as a powerful stimulator of the MLR had not been appreciated during the 15 years in which this test had been in active use. These observations led us to suggest in 1978 that "DC will prove to be a critical accessory cell required in the generation of many immune responses" [22].

When the 33D1 mAb was developed by Michel Nussenzweig in 1982 [16], selective removal of the tiny fraction of DC became feasible. The absence of DC ablated much of the MLR stimulating activity from the total suspension of spleen cells [17]. We could not interpret the residual activity in 33D1-depleted spleen cells until 7 years later, when we noted that there were two subsets of DC in mouse spleen, a major $33D1^{+}$ and a minor NLDC-145^{+} (now DEC-205/$CD205^{+}$) subset [30]. These subsets were denoted as $CD8^{-}$ and $CD8^{+}$ by Ken Shortman [31]. Because we could selectively deplete most of the MLR stimulating function by removal of DC from a fresh spleen cell suspension, we concluded that "antigens, to be immunogens, require accessory cells" [17].

Robert Lechler and Richard Batchelor did a pivotal transplant experiment *in vivo*. They first induced tolerance of F1 rat kidneys in a parental strain, and then they injected different types of F1 cells to try to break tolerance or induce kidney rejection. F1 DC in small numbers (10^4–10^5 per rat) induced rejection, whereas $>10^6$ lymphocytes were inactive [32]. We worked with Denise Faustman *et al.* to treat pancreatic islets with the 33D1 mAb and complement, which selectively eliminated the few DC that could be visualized in each islet. The depleted islets could then be transplanted across an MHC barrier [33]. In effect, DC were converting transplantation antigens, *i.e*. MHC products, into immunogens both in tissue culture and in animals. DC were needed not only for presentation of MHC products, or immune recognition, but as potent accessories for *immune responsiveness*.

Dendritic cells as antigen-presenting cells for autologous T cells

To analyze the capacity of DC to induce responses from autologous T cells, Nussenzweig used a system described by Gene Shearer, who had discovered that the hapten TNP was presented to $CD8^{+}$ killer T cells in an MHC-restricted manner. Nussenzweig prepared TNP-coated T cells, which were not themselves immunogenic, and added them to cultures of different antigen-presenting cells and T cells. Only DC could initiate $CD8^{+}$ T cell immunity to TNP [34]. In retrospect, this was likely the first example of the powerful cross-presenting function of DC, in which the cells capture nonreplicating antigens and present these on MHC I. In the first review on DC in 1980, Michel and I [35] concluded "DC must belong to a separate, bone marrow-derived lineage. They exhibit distinct cytological features, surface markers and functional capacities, all of which are stable in tissue culture".

To prove that DC were required to immunize helper T cells, Kayo Inaba examined the antibody response. During her PhD thesis research in Kyoto, she had realized that some cell other than an Fc receptor-rich macrophage was critical for antibody formation in Mishell Dutton cultures [36]. We joined forces after a meeting sponsored by the Naito Foundation in Tokyo in 1980. Purified DC proved to be potent accessories for antibody formation, while macrophages were weak. We also depleted fresh spleen cells of the trace DC component with 33D1 mAb and complement. Much of the antibody response in an otherwise unmanipulated spleen suspension was ablated, and could be restored by small numbers of enriched DC [37]. We went on to use DC to prime helper T cells to carrier proteins like KLH and HSA [38]. Again DC were required to induce (prime, sensitize) helper T cells in the afferent limb of the antibody response, while in the *efferent* limb, newly differentiated T cells acted on other antigen-presenting cells, here B cells, to provide the help required for antibody formation. Similar results were obtained in other systems involving the killing of targets and the activation of macrophages [39, 40], *i.e*. DC first activated killer and helper T cells, and then these interacted in the efferent limb with other antigen presenting cells. Thus, immune responses involved afferent and efferent limbs, each requiring antigen presentation and MHC restriction.

Other labs were obtaining evidence for cells distinct from macrophages that had active accessory function, usually with polyclonal mitogens as the T cell stimulus. William Bowers [41, 42] and Don Mason and Gordon MacPherson [25, 43] found active nonadherent accessory cells in thoracic duct lymph of rats, while Brigid Balfour, Hemmo Drexhage and Stella Knight [44–46] described similar veiled cells in the afferent lymph of rabbits and guinea pigs. These early observations on distinct cells in different tissues and species were brought together in a review in 1982, where ideas of a DC system were considered [47].

Langerhans cells as immature dendritic cells

We then turned to peripheral tissues to determine if we could identify and characterize DC. Skin was chosen because several other investigators had discovered that Langerhans cells (LC) in the epidermis were leukocytes

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that expressed MHC class II and could present antigens. Experiments on the skin became feasible with the arrival in our lab of Gerold Schuler, a dermatologist from a leading research group in Tyrol with expertise in LC. He discovered a pivotal feature of immunogenicity, DC maturation. Surprisingly, he found that LC, in spite of their abundant MHC class II products, were poorly immunogenic in the MLR and mitogen assays [48]. Instead, the LC needed to be cultured to differentiate and become potent immunogens [48, 49]. When the LC were purified, it became evident that granulocyte macrophage stimulating factor (GM-CSF) was essential for LC maturation (Fig. 2) [50]. Then specific protein antigens were tested by another Tyrolean, Nikolaus Romani, who made a second striking observation. The freshly isolated LC could present antigen, although only for a short time, while the mature cultured ones did not. In contrast, the mature cultured DC were powerful accessories for immunity to previously captured antigens. Thus maturation involved two steps that lead to T cell recognition (antigen processing and peptide-MHC formation) and responsiveness (often termed costimulation), and these two distinct aspects of immunogenicity could be separated in time.

Ira Mellman, Antonio Lanzavecchia and their colleagues later showed what was going on at the level of antigen presentation in maturing DC (reviewed in [51]). Immature DC were endocytic and much of the cell's MHC II products were inside the cell [52, 53]. Maturation eventually led to a dampening of endocytosis, accounting for the weak endocytic activity that had been observed years before. Maturation also activated the processing machinery in late endosomes or lysosomes, and allowed for increased formation of peptide-MHC complexes [54]. This was followed by transport of peptide-MHC to the cell surface [55] where degradation was avoided, probably by a newly recognized control in which mature DC turn off ubiquitination of MHC II [56]. Maturation, the differentiation of DC in response to environmental stimuli, is a key to immunogenicity and can involve hundreds of changes in response to signals from microbes, immune complexes, cytokines, innate lymphocytes and a variety of endogenous stimuli currently called "danger signals" or "alarmins". Different maturation programs essentially allow DC to control the distinct qualities of the subsequent lymphocyte responses.

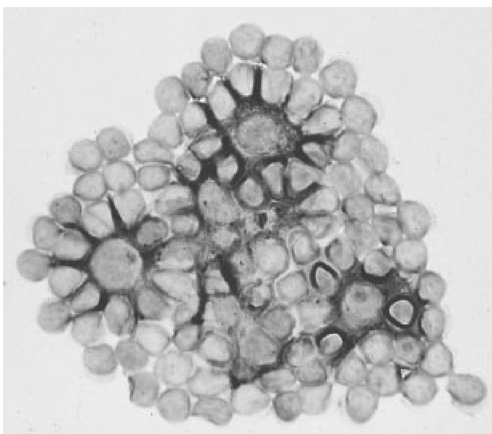

Figure 2. Clusters of dendritic cells (dark stellate profiles) and T lymphocytes, a microenvironment for gererating immunity. Courtesy of N Romani. The figure is reproduced with permission from [99].

Dendritic cells as nature's adjuvants *in vivo*

Along with Josh Metlay, an MD-PhD student, Inaba and I [57] turned to foreign proteins to assess whether DC could serve as "nature's adjuvants" for immunogenicity *in vivo*. We exposed immature DC to different proteins, allowed the cells to differentiate in culture overnight, and injected the antigen-charged cells into mice. The T cells were immunized and restricted to the MHC products of the injected DC. In other words, without any Freund's or alum adjuvant, DC initiated immunity *in vivo*.

An important advance was to learn how to generate DC from progenitors, which became possible in several labs in the 1990's [58–62]. This made DC much more accessible for research. The newly generated cells proved to be immunogenic *in vivo* [63]. Understanding the development of DC, including their various forms or subsets with different markers and functions, remains a major field.

Several scientists then used DC as adjuvants in humans [64–67]. Controlling DC maturation is likely to be an important component of this approach [68, 69] and requires studies in patients to analyze properly. "Active" immunotherapy with DC, which aims to stimulate patients to develop their own immunity to cancer, has a major limitation currently. It is that the injected DC migrate and persist poorly in the recipient lymphoid tissues, thereby failing to reproduce a critical homing function of DC for immunogenicity. The use of *ex vivo* derived DC in immunotherapy is currently an understudied but valuable approach to probe the human immune response, particularly if one could overcome this major migration obstacle and also garner support for a more vigorous and better coordinated research effort.

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Tolerogenicity, another side of immunogenicity

The function of DC in tolerance emerged when antigens were first selectively targeted to DC *in situ* by Nussenzweig and by Inaba. To do so, Nussenzweig engineered antigens into the anti-DEC-205 mAb [70, 71], which recognized an endocytic receptor [72] expressed by many DC in the T cell areas of lymphoid tissues [73]. Additionally, Inaba studied antigens within dying cells [74]. These experiments allowed one to probe DC function directly *in situ*, without having to isolate the cells. Following both forms of antigen targeting to DC *in vivo* in the steady state, *i.e*. using the hybrid antibodies or dying cells, antigen presentation took place but the corresponding T cells were deleted. When a stimulus for DC maturation was coadministered with the targeted antigen, clonal expansion was accompanied by differentiation to high levels of IFN-γ production [70, 71, 75]. As a result, we proposed that DC could control both immunity and tolerance.

The role of DC as tolerogens is thought to be essential to balance their role as immunogens, because when DC capture dying infected cells and mature, they simultaneously present microbial antigens as well as harmless self and environmental antigens [76, 77]. Through tolerance, DC orchestrate the lymphocyte repertoire to dampen the danger of subsequent self reactivity. Then, when infection strikes, maturing DC focus immunity to the microbe.

A newly discovered facet of tolerance by DC is that they induce suppressor T cells, which are often termed "T regs" by the research community. DC can differentiate T regs in the thymus [78] and in the periphery [79], and expand them once the T regs have formed [80–82]. Antigen-specific T regs are much more effective than polyclonal populations in suppressing immunity *in vivo* [79, 81–83]. For many years, the major approach to the induction of peripheral tolerance has been to inject very large doses of antigen. Nowadays, the efficient and more physiologic induction of antigen-specific tolerance is becoming feasible by directing antigens to DC and controlling their maturation state.

New developments in antigen handling and maturation of dendritic cells, two critical aspects of immunogenicity

Antigen handling, *i.e*. capture, processing and presentation, is a key step in the antigen-specific control of immunity and tolerance, but for many years, antigen capture was not manipulated directly. Instead, sizeable doses of antigen were administered to reveal the effective antigen processing capacities of DC *in vivo*, *e.g*. [84]. It is now evident that DC express many potential receptors for antigen uptake, including the first two markers recognized by anti-DC antibodies, 33D1 to DCIR2 [18], and NLDC-145 to DEC-205 [72, 85]. These receptors are expressed by distinct subsets of DC in the mouse. When antigen is incorporated into these antibodies and targeted to the corresponding receptors in mice [18, 70, 86–90], presentation is increased more than 100-fold. Reciprocally, if one simply injects nontargeted antigen, one is under-exploiting the capacity of DC to control immunity and tolerance by 100-fold. DC also take up dying cells *in vivo*, including tumor cells [74, 91–93], and this likewise greatly increases the efficiency of antigen presentation, although the receptors for uptake remain unidentified. In both instances, uptake of DEC-205 ligands and dying cells, DC are able to cross-present antigens on MHC class I products to $CD8^+$ T cells. A fascinating recent example has been provided in the case of HIV gag protein delivered within antibodies to human DEC-205. For the first time, it has been shown that cross-presentation is able to extract defined peptides for presentation to $CD8^+$ T cells across a spectrum of MHC haplotypes [94]. I am excited about this new field of receptor-based antigen targeting to DC, which allows one to analyze DC function – both antigen processing and subsequent interactions with lymphocytes – directly *in vivo*, without the need for cell isolation.

Another critical component of immunogenicity, DC maturation, also needs to be pursued to identify the molecular mechanisms that maturing DC utilize to differentiate T cells along one pathway or another. Maturation, the stimulus-dependent differentiation of DC that leads to different types of immunity and tolerance, allows DC to tailor the immune response so that it is appropriate to the pathogen or other stimulus. Maturation entails the expression of numerous families of membrane-associated costimulatory molecules, cytokines, chemokines and other active metabolites. It has become clear that a traditional model, *i.e*. signal one (peptide-MHC) and signal two (B7 costimulators), is insufficient to explain immunogenicity, because DC are competent in both and can still require additional functions, *e.g*. through CD40 signals [75]. Recently, it was found that the up-regulation of CD70 was essential for one subset of $DEC\text{-}205^+$ DC to induce the Th1 pathway of $CD4^+$ T cell differentiation [90]. The function of DC subsets is not emphasized here, but clearly, subsets differ in their expression of uptake receptors, processing capacities, and responses to maturation stimuli. By understanding and controlling antigen handling and maturation of DC and their subsets *in vivo*, it should be possible to better control antigen-specific immune resistance and silencing.

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Dendritic cells as a pivotal force in medicine

Immunology is pivotal to major problems in medicine, which are also increasing in prevalence. Many clinically relevant antigens are known and/or being identified, but it is still necessary to uncover the required pathways for immunogenicity and tolerogenicity, *i.e*. to identify antigen-specific therapies that can either enhance (as in infection and cancer) or dampen (as in autoimmunity, allergy, and transplantation) the immune response. The knowledge derived from DC biology about innate and adaptive immune responses, and about their roles in medicine, helps one to think more concretely about the specific driving forces for disease, both pathogenesis and the design of preventions and treatments, *e.g*. in HIV-1 infection [88, 95, 96].

Regrettably, research on patients remains an under-represented field, one that suffers from many obstacles [97]. This lack of activity occurs despite strong evidence that antibody-based, immune therapies are the most successful and prominent new therapy for cancer and other diseases, and that cell-mediated immunity also has significant potential to provide nontoxic and highly specific treatments. We need to build a new research enterprise, without damaging the existing one, to breed investigators who study the immunology of disease in humans and to provide them with a supportive culture and research funds.

Dendritic cells as part of the tapestry of immunology and medicine

Many threads of research by scientists from many countries contributed to the discovery of the DC system [98]. This personal article explains some of the experiments that we did, why we did them and our interpretations of the findings. However, our discipline is like a tapestry, because immunology uses different sources, colors and weaves of materials to create an array of beautiful and valuable products. This was certainly the case for all the events related in this summary. At each point, we depended on reagents and discoveries from other distinct areas of research. The rich tapestry of immunology and biology has allowed the theme of immunogenicity to expand its impact and to identify new mechanisms. I am grateful to the National Institutes of Health and to several foundations for research support and to the Rockefeller University for support and stimulating traditions in cell biology, patient-based research, and immunology.

References

1 **Sela, M.,** Antigenicity: some molecular aspects. *Science* 1969. **166:** 1365–1374.

2 **McDevitt, H. O. and Sela, M.,** Genetic control of the antibody response. I. Demonstration of determinant-specific differences in response to synthetic polypeptide antigens in two strains of inbred mice. *J. Exp. Med.* 1965. **122:** 517–531.

3 **McDevitt, H. O., Deak, B. D., Shreffler, D. C., Klein, J., Stimpfling, J. H. and Snell, G. D.,** Genetic control of the immune response. Mapping of the Ir-1 locus. *J. Exp. Med.* 1972. **135:** 1259–1278.

4 **Nossal, G. J. V., Abbot, A., Mitchell, J. and Lummus, Z.,** Antigen in immunity. XV. Ultrastructural features of antigen capture in primary and secondary lymphoid follicles. *J. Exp. Med.* 1968. **127:** 277–296.

5 **White, R. G., French, V. I. and Stark, J. M.,** A study of the localization of a protein antigen in the chicken spleen and its relation to the formation of germinal centers. *J. Med. Microbiol.* 1970. **3:** 65–83.

6 **Unanue, E. R. and Cerottini, J.-C.,** The immunogenicity of antigen bound to the plasma membrane of macrophages. *J. Exp. Med.* 1970. **131:** 711–726.

7 **Steinman, R. M. and Cohn, Z. A.,** The interaction of soluble horseradish peroxidase with mouse peritoneal macrophages *in vitro*. *J. Cell Biol.* 1972. **55:** 186–204.

8 **Steinman, R. M. and Cohn, Z. A.,** The interaction of particulate horseradish peroxidase (HRP)-anti HRP immune complexes with mouse peritoneal macrophages *in vitro*. *J. Cell Biol.* 1972. **55:** 616–634.

9 **Steinman, R. M., Brodie, S. E. and Cohn, Z. A.,** Membrane flow during pinocytosis. A stereologic analysis. *J. Cell Biol.* 1976. **68:** 665–687.

10 **Steinman, R. M., Mellman, I. S., Muller, W. A. and Cohn, Z. A.,** Endocytosis and the recycling of plasma membrane. *J. Cell Biol.* 1983. **96:** 1–27.

11 **Goldstein, J. L., Anderson, R. G. and Brown, M. S.,** Coated pits, coated vesicles, and receptor-mediated endocytosis. *Nature* 1979. **279:** 679–685.

12 **Mishell, R. I. and Dutton, R. W.,** Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* 1967. **126:** 423–442.

13 **Steinman, R. M. and Cohn, Z. A.,** Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J. Exp. Med.* 1973. **137:** 1142–1162.

14 **Steinman, R. M. and Cohn, Z. A.,** Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties *in vitro*. *J. Exp. Med.* 1974. **139:** 380–397.

15 **Steinman, R. M., Kaplan, G., Witmer, M. D. and Cohn, Z. A.,** Identification of a novel cell type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance in vitro. *J. Exp. Med.* 1979. **149:** 1–16.

16 **Nussenzweig, M. C., Steinman, R. M., Witmer, M. D. and Gutchinov, B.,** A monoclonal antibody specific for mouse dendritic cells. *Proc. Natl. Acad. Sci. USA* 1982. **79:** 161–165.

17 **Steinman, R. M., Gutchinov, B., Witmer, M. D. and Nussenzweig, M. C.,** Dendritic cells are the principal stimulators of the primary mixed leukocyte reaction in mice. *J. Exp. Med.* 1983. **157:** 613–627.

18 **Dudziak, D., Kamphorst, A. O., Heidkamp, G. F., Buchholz, V., Trumpfheller, C., Yamazaki, S., Cheong, C. *et al.*,** Differential antigen processing by dendritic cell subsets *in vivo*. *Science* 2007. **315:** 107–111.

19 **Crowley, M. T., Inaba, K., Witmer-Pack, M. D., Gezelter, S. and Steinman, R. M.,** Use of the fluorescence activated cell sorter to enrich dendritic cells from mouse spleen. *J. Immunol. Meth.* 1990. **133:** 55–66.

20 **Metlay, J. P., Witmer-Pack, M. D., Agger, R., Crowley, M. T., Lawless, D. and Steinman, R. M.,** The distinct leukocyte integrins of mouse spleen dendritic cells as identified with new hamster monoclonal antibodies. *J. Exp. Med.* 1990. **171:** 1753–1771.

21 **Lindquist, R. L., Shakhar, G., Dudziak, D., Wardemann, H., Eisenreich, T., Dustin, M. L. and Nussenzweig, M. C.,** Visualizing dendritic cell networks *in vivo*. *Nat. Immunol.* 2004. **5:** 1243–1250.

22 **Steinman, R. M. and Witmer, M. D.,** Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. *Proc. Natl. Acad. Sci. USA* 1978. **75:** 5132–5136.

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23 **Bain, B., Vas, M. R. and Lowenstein, L.,** The development of large immature mononuclear cells in mixed leukocyte cultures. *Blood* 1964. **23:** 108–116.

24 **Bach, F. and Hirschhorn, K.,** Lymphocyte interaction: A potential histocompatibility test *in vitro*. *Science* 1964. **143:** 813–814.

25 **Mason, D. W., Pugh, C. W. and Webb, M.,** The rat mixed lymphocyte reaction: roles of a dendritic cell in intestinal lymph and T cell subsets defined by monoclonal antibodies. *Immunology* 1981. **44:** 75–87.

26 **Van Voorhis, W. C., Hair, L. S., Steinman, R. M. and Kaplan, G.,** Human dendritic cells. Enrichment and characterization from peripheral blood. *J. Exp. Med.* 1982. **155:** 1172–1187.

27 **Inaba, K. and Steinman, R. M.,** Resting and sensitized T lymphocytes exhibit distinct stimulatory [antigen-presenting cell] requirements for growth and lymphokine release. *J. Exp. Med.* 1984. **160:** 1717–1735.

28 **Inaba, K., Witmer, M. D. and Steinman, R. M.,** Clustering of dendritic cells, helper T lymphocytes, and histocompatible B cells, during primary antibody responses *in vitro*. *J. Exp. Med.* 1984. **160:** 858–876.

29 **Inaba, K. and Steinman, R. M.,** Accessory cell - T lymphocyte interactions: antigen dependent and independent clustering. *J. Exp. Med.* 1986. **163:** 247–261.

30 **Crowley, M., Inaba, K., Witmer-Pack, M. and Steinman, R. M.,** The cell surface of mouse dendritic cells: FACS analyses of dendritic cells from different tissues including thymus. *Cell. Immunol.* 1989. **118:** 108–125.

31 **Vremec, D., Zorbas, M., Scollay, R., Saunders, D. J., Ardavin, C. F., Wu, L. and Shortman, K.,** The surface phenotype of dendritic cells purified from mouse thymus and spleen: investigation of the CD8 expression by a subpopulation of dendritic cells. *J. Exp. Med.* 1992. **176:** 47–58.

32 **Lechler, R. I. and Batchelor, J. R.,** Restoration of immunogenicity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. *J. Exp. Med.* 1982. **155:** 31–41.

33 **Faustman, D. L., Steinman, R. M., Gebel, H. M., Hauptfeld, V., Davie, J. M. and Lacy, P. E.,** Prevention of rejection of murine islet allografts by pretreatment with anti-dendritic cell antibody. *Proc. Natl. Acad. Sci. USA* 1984. **81:** 3864–3868.

34 **Nussenzweig, M. C., Steinman, R. M., Gutchinov, B. and Cohn, Z. A.,** Dendritic cells are accessory cells for the development of anti-trinitrophenyl cytotoxic T lymphocytes. *J. Exp. Med.* 1980. **152:** 1070–1084.

35 **Steinman, R. M. and Nussenzweig, M. C.,** Dendritic cells: features and functions. *Immunol. Rev.* 1980. **53:** 127–147.

36 **Inaba, K., Nakano, K. and Muramatsu, S.,** Cellular synergy in the manifestation of accessory cell activity for *in vitro* antibody response. *J. Immunol.* 1981. **127:** 453–461.

37 **Inaba, K., Steinman, R. M., Van Voorhis, W. C. and Muramatsu, S.,** Dendritic cells are critical accessory cells for thymus-dependent antibody responses in mouse and man. *Proc. Natl. Acad. Sci. USA* 1983. **80:** 6041–6045.

38 **Inaba, K. and Steinman, R. M.,** Protein-specific helper T lymphocyte formation initiated by dendritic cells. *Science* 1985. **229:** 475–479.

39 **Inaba, K., Young, J. W. and Steinman, R. M.,** Direct activation of $CD8^+$ cytotoxic T lymphocytes by dendritic cells. *J. Exp. Med.* 1987. **166:** 182-–94.

40 **Koide, S. and Steinman, R. M.,** Induction of interleukin-1α mRNA during the antigen-dependent interaction of sensitized T lymphoblasts with macrophages. *J. Exp. Med.* 1988. **168:** 409–416.

41 **Klinkert, W. E. F., Labadie, J. H., O'Brien, J. P., Beyer, C. F. and Bowers, W. E.,** Rat dendritic cells function as accessory cells and control the production of a soluble factor required for mitogen responses of T lymphocytes. *Proc. Natl. Acad. Sci. USA* 1978. **77:** 5414–5418.

42 **Klinkert, W. E. F., Labadie, J. H. and Bowers, W. E.,** Accessory and stimulating properties of dendritic cells and macrophages isolated from various rat tissues. *J. Exp. Med.* 1982. **156:** 1–19.

43 **Pugh, C. W., MacPherson, G. G. and Steer, H. W.,** Characterization of nonlymphoid cells derived from rat peripheral lymph. *J. Exp. Med.* 1983. **157:** 1758–1779.

44 **Knight, S. C., Balfour, B. M., O'Brien, J., Buttifant, L., Sumerska, T. and Clark, J.,** Role of veiled cells in lymphocyte activation. *Eur. J. Immunol.* 1982. **12:** 1057–1060.

45 **Kelly, R. H., Balfour, B. M., Armstrong, J. A. and Griffiths, S.,** Functional anatomy of lymph nodes. II. Peripheral lymph-borne mononuclear cells. *Anat. Rec.* 1978. **190:** 5–21.

46 **Drexhage, H. A., Mullink, H., de Groot, J., Clarke, J. and Balfour, B. M.,** A study of cells present in peripheral lymph of pigs with special reference to a type of cell resembling the Langerhans cells. *Cell. Tiss. Res.* 1979. **202:** 407–430.

47 **Tew, J. G., Thorbecke, J. and Steinman, R. M.,** Dendritic cells in the immune response: characteristics and recommended nomenclature. *J. Reticuloendothel.Soc.* 1982. **31:** 371–380.

48 **Schuler, G. and Steinman, R. M.,** Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells *in vitro*. *J. Exp. Med.* 1985. **161:** 526–546.

49 **Inaba, K., Schuler, G., Witmer, M. D., Valinsky, J., Atassi, B. and Steinman, R. M.,** The immunologic properties of purified Langerhans cells: distinct requirements for the stimulation of unprimed and sensitized T lymphocytes. *J. Exp. Med.* 1986. **164:** 605–613.

50 **Witmer-Pack, M. D., Olivier, W., Valinsky, J., Schuler, G. and Steinman, R. M.,** Granulocyte/macrophage colony-stimulating factor is essential for the viability and function of cultured murine epidermal Langerhans cells. *J. Exp. Med.* 1987. **166:** 1484–1498.

51 **Trombetta, E. S. and Mellman, I.,** Cell biology of antigen processing *in vitro* and *in vivo*. *Annu. Rev. Immunol.* 2005. **23:** 975–1028.

52 **Pierre, P., Turley, S. J., Gatti, E., Hull, M., Meltzer, J., Mirza, A., Inaba, K. *et al.*,** Developmental regulation of MHC class II transport in mouse dendritic cells. *Nature* 1997. **388:** 787–792.

53 **Cella, M., Engering, A., Pinet, V., Pieters, J. and Lanzavecchia, A.,** Inflammatory stimuli induce accumulation of MHC class II complexes on dendritic cells. *Nature* 1997. **388:** 782–787.

54 **Inaba, K., Turley, S., Iyoda, T., Yamaide, F., Shimoyama, S., Reis e Sousa, C., Germain, R. N., Mellman, I. and Steinman, R. M.,** The formation of immunogenic MHC class II- peptide ligands in lysosomal compartments of dendritic cells is regulated by inflammatory stimuli. *J. Exp. Med.* 2000. **191:** 927–936.

55 **Turley, S. J., Inaba, K., Garrett, W. S., Ebersold, M., Untermaehrer, J., Steinman, R. M. and Mellman, I.,** Transport of peptide-MHC class II complexes in developing dendritic cells. *Science* 2000. **288:** 522–527.

56 **Shin, J. S., Ebersold, M., Pypaert, M., Delamarre, L., Hartley, A. and Mellman, I.,** Surface expression of MHC class II in dendritic cells is controlled by regulated ubiquitination. *Nature* 2006. **444:** 115–118.

57 **Inaba, K., Metlay, J. P., Crowley, M. T. and Steinman, R. M.,** Dendritic cells pulsed with protein antigens in vitro can prime antigen-specific, MHC-restricted T cells *in situ*. *J. Exp. Med.* 1990. **172:** 631–640.

58 **Inaba, K., Inaba, M., Romani, N., Aya, H., Deguchi, M., Ikehara, S., Muramatsu, S. and Steinman, R. M.,** Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 1992. **176:** 1693–1702.

59 **Inaba, K., Steinman, R. M., Witmer-Pack, M., Aya, H., Inaba, M., Sudo, T. *et al.*,** Identification of proliferating dendritic cell precursors in mouse blood. *J. Exp. Med.* 1992. **175:** 1157–1167.

60 **Caux, C., Dezutter-Dambuyant, C., Schmitt, D. and Banchereau, J.,** GM-CSF and TNF-α cooperate in the generation of dendritic Langerhans cells. *Nature* 1992. **360:** 258–261.

61 **Romani, N., Gruner, S., Brang, D., Kämpgen, E., Lenz, A., Trockenbacher, B., Konwalinka, G., Fritsch, P. O., Steinman, R. M. and Schuler, G.,** Proliferating dendritic cell progenitors in human blood. *J. Exp. Med.* 1994. **180:** 83–93.

62 **Sallusto, F. and Lanzavecchia, A.,** Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *J. Exp. Med.* 1994. **179:** 1109–1118.

63 **Inaba, K., Inaba, M., Naito, M. and Steinman, R. M.,** Dendritic cell progenitors phagocytose particulates, including Bacillus Calmette-Guerin organisms, and sensitize mice to mycobacterial antigens *in vivo*. *J. Exp. Med.* 1993. **178:** 479–488.

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64 **Nestle, F. O., Alijagic, S., Gilliet, M., Sun, Y., Grabbe, S., Dummer, R., Burg, G. and Schadendorf, D.,** Vaccination of melanoma patients with peptide-or tumor lysate-pulsed dendritic cells. *Nat. Med.* 1998. **4:** 328–332.

65 **Hsu, F. J., Benike, C., Fagnoni, F., Liles, T. M., Czerwinski, D., Taidi, B., Engleman, E. G. and Levy, R.,** Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nat. Med.* 1996. **2:** 52–58.

66 **Dhodapkar, M., Steinman, R. M., Sapp, M., Desai, H., Fossella, C., Krasovsky, J., Donahoe, S. M. *et al.*,** Rapid generation of broad T-cell immunity in humans after single injection of mature dendritic cells. *J. Clin. Invest.* 1999. **104:** 173–180.

67 **Thurner, B., Haendle, I., Röder, C., Dieckmann, D., Keikavoussi, P., Jonuleit, H., Bender, A. *et al.*,** Vaccination with Mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. *J. Exp. Med.* 1999. **190:** 1669–1678.

68 **Bender, A., Sapp, M., Schuler, G., Steinman, R. M. and Bhardwaj, N.,** Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood. *J. Immunol. Methods* 1996. **196:** 121–135.

69 **Romani, N., Reider, D., Heuer, M., Ebner, S., Eibl, B., Niederwieser, D. and Schuler, G.,** Generation of mature dendritic cells from human blood: an improved method with special regard to clinical applicability. *J. Immunol. Methods* 1996. **196:** 137–151.

70 **Hawiger, D., Inaba, K., Dorsett, Y., Guo, K., Mahnke, K., Rivera, M., Ravetch, J. V. *et al.*,** Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions *in vivo*. *J. Exp. Med.* 2001. **194:** 769–780.

71 **Bonifaz, L., Bonnyay, D., Mahnke, K., Rivera, M., Nussenzweig, M. C. and Steinman, R. M.,** Efficient targeting of protein antigen to the dendritic cell receptor DEC-205 in the steady state leads to antigen presentation on major histocompatibility complex class I products and peripheral $CD8^+$ T cell tolerance. *J. Exp. Med.* 2002. **196:** 1627–1638.

72 **Jiang, W., Swiggard, W. J., Heufler, C., Peng, M., Mirza, A., Steinman, R. M. and Nussenzweig, M. C.,** The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing. *Nature* 1995. **375:** 151–155.

73 **Kraal, G., Breel, M., Janse, M. and Bruin, G.,** Langerhans cells, veiled cells, and interdigitating cells in the mouse recognized by a monoclonal antibody. *J. Exp. Med.* 1986. **163:** 981–997.

74 **Liu, K., Iyoda, T., Saternus, M., Kimura, K., Inaba, K. and Steinman, R. M.,** Immune tolerance after delivery of dying cells to dendritic cells *in situ*. *J. Exp. Med.* 2002. **196:** 1091–1097.

75 **Fujii, S., Liu, K., Smith, C., Bonito, A. J. and Steinman, R. M.,** The linkage of innate to adaptive immunity *via* maturing dendritic cells *in vivo* requires CD40 ligation in addition to antigen presentation and CD80/86 costimulation. *J. Exp. Med.* 2004. **199:** 1607–1618.

76 **Steinman, R. M., Turley, S., Mellman, I. and Inaba, K.,** The induction of tolerance by dendritic cells that have captured apoptotic cells. *J. Exp. Med.* 2000. **191:** 411–416.

77 **Steinman, R. M. and Nussenzweig, M. C.,** Avoiding horror autotoxicus: the importance of dendritic cells in peripheral T cell tolerance. *Proc. Natl. Acad. Sci. USA* 2002. **99:** 351–358.

78 **Watanabe, N., Wang, Y. H., Lee, H. K., Ito, T., Cao, W. and Liu, Y. J.,** Hassall's corpuscles instruct dendritic cells to induce $CD4^+CD25^+$ regulatory T cells in human thymus. *Nature* 2005. **436:** 1181–1185.

79 **Luo, X., Tarbell, K. V., Yang, H., Pothoven, K., Bailey, S. L., Ding, R., Steinman, R. M. and Suthanthiran, M.,** Dendritic cells with TGF-β1 differentiate naive $CD4^+CD25^-$ T cells into islet-protective $Foxp3^+$ regulatory T cells. *Proc. Natl. Acad. Sci. USA* 2007. **104:** 2821–2826.

80 **Yamazaki, S., Iyoda, T., Tarbell, K., Olson, K., Velinzon, K., Inaba, K. and Steinman, R. M.,** Direct expansion of functional $CD25^+$ $CD4^+$ regulatory T cells by antigen processing dendritic cells. *J. Exp. Med.* 2003. **198:** 235–247.

81 **Tarbell, K. V., Yamazaki, S., Olson, K., Toy, P. and Steinman, R. M.,** $CD25^+$ $CD4^+$ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J. Exp. Med.* 2004. **199:** 1467–1477.

82 **Yamazaki, S., Patel, M., Harper, A., Bonito, A., Fukuyama, H., Pack, M., Tarbell, K. V. *et al.*,** Effective expansion of alloantigen-specific *Foxp3*$^+$ $CD25^+$ regulatory T cells by dendritic cells during the mixed leukocyte reaction. *Proc. Natl. Acad. Sci. USA* 2006. **103:** 2758–2763.

83 **Tarbell, K. V., Petit, L., Zuo, X., Toy, P., Luo, X., Mqadmi, A., Yang, H. *et al.*,** Dendritic cell-expanded, islet-specific, $CD4^+$ $CD25^+$ $CD62L^+$ regulatory T cells restore normoglycemia in diabetic NOD mice. *J. Exp .Med.* 2007. **204:** 191–201.

84 **Crowley, M., Inaba, K. and Steinman, R. M.,** Dendritic cells are the principal cells in mouse spleen bearing immunogenic fragments of foreign proteins. *J. Exp. Med.* 1990. **172:** 383–386.

85 **Mahnke, K., Guo, M., Lee, S., Sepulveda, H., Swain, S. L., Nussenzweig, M. and Steinman, R. M.,** The dendritic cell receptor for endocytosis, DEC-205, can recycle and enhance antigen presentation *via* major histocompatibility complex class II-positive lysosomal compartments. *J. Cell Biol.* 2000. **151:** 673–683.

86 **Bonifaz, L. C., Bonnyay, D. P., Charalambous, A., Darguste, D. I., Fujii, S., Soares, H., Brimnes, M. K. *et al.*,** *In vivo* targeting of antigens to maturing dendritic cells *via* the DEC-205 receptor improves T cell vaccination. *J. Exp. Med.* 2004. **199:** 815–824.

87 **Boscardin, S. B., Hafalla, J. C., Masilamani, R. F., Kamphorst, A. O., Zebroski, H. A., Rai, U., Morrot, A. *et al.*,** Antigen targeting to dendritic cells elicits long-lived T cell help for antibody responses. *J. Exp. Med.* 2006. **203:** 599–606.

88 **Trumpfheller, C., Finke, J. S., Lopez, C. B., Moran, T. M., Moltedo, B., Soares, H., Huang, Y. *et al.*,** Intensified and protective $CD4^+$ T cell immunity at a mucosal surface after a single dose of anti-dendritic cell HIV gag fusion antibody vaccine. *J. Exp. Med.* 2006. **203:** 607–617.

89 **Charalambous, A., Oks, M., Nchinda, G., Yamazaki, S. and Steinman, R. M.,** Dendritic cell targeting of survivin protein in a xenogeneic form elicits strong $CD4^+$ T cell immunity to mouse survivin. *J. Immunol.* 2006. **177:** 8410–8421.

90 **Soares, H., Waechter, H., Glaichenhaus, N., Mougneau, E., Yagita, H., Mizenina, O., Dudziak, D. *et al.*,** DEC-205/$CD205^+$ dendritic cells induce $CD4^+$ T cells to produce IFN-γ by a CD70 dependent, IL-12 independent mechanism. *J. Exp. Med.* 2007. **204:** 1095–1106.

91 **Iyoda, T., Shimoyama, S., Liu, K., Omatsu, Y., Maeda, Y., Takahara, K., Akiyama, Y. *et al.*,** The $CD8^+$ dendritic cell subset selectively endocytoses dying cells in culture and *in vivo*. *J. Exp. Med.* 2002. **195:** 1289–1302.

92 **Fujii, S., Shimizu, K., Smith, C., Bonifaz, L. and Steinman, R. M.,** Activation of natural killer T cells by α-galactosylceramide rapidly induces the full maturation of dendritic cells *in vivo* and thereby acts as an adjuvant for combined CD4 and CD8 T cell immunity to a co-administered protein. *J. Exp. Med.* 2003. **198:** 267–279.

93 **Liu, K., Idoyaga, J., Charalambous, A., Fujii, S., Bonito, A., Mordoh, J., Wainstok, R. *et al.*,** Innate NKT lymphocytes confer superior adaptive immunity *via* tumor-capturing dendritic cells. *J. Exp. Med.* 2005. **202:** 1507–1516.

94 **Bozzacco, L., Trumpfheller, C., Siegal, F. P., Mehandru, S., Markowitz, M., Carrington, M., Nussenzweig, M. C. *et al.*,** DEC-205 receptor on dendritic cells mediates presentation of HIV gag protein to $CD8^+$ T cells in a spectrum of human MHC I haplotypes. *Proc. Natl. Acad. Sci. USA* 2007. **104:** 1289–1294

95 **Cameron, P. U., Freudenthal, P. S., Barker, J. M., Gezelter, S., Inaba, K. and Steinman, R. M.,** Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to $CD4^+$ T cells. *Science* 1992. **257:** 383–387.

96 **Pope, M., Betjes, M. G. H., Romani, N., Hirmand, H., Cameron, P. U., Hoffman, L., Gezelter, S. *et al.*,** Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. *Cell* 1994. **78:** 389–398.

97 **Steinman, R. M. and Mellman, I.,** Immunotherapy: bewitched, bothered, and bewildered no more. *Science* 2004. **305:** 197–200.

98 **Steinman, R. M.,** Dendritic cells: from the fabric of immunology. *Clin. Invest. Med.* 2004. **27:** 231–236.

99 **Inaba, K., Romani, N. and Steinman, R. M.,** An antigen-independent contact mechanism as an early step in T cell-proliferative responses to dendritic cells. *J. Exp. Med.* 1989. **170:** 527–542.

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