Dendritic Cells: Immune Activators or Virus Facilitators?

Melissa Pope, PhD
Scientist, Center for Biomedical Research
Population Council, New York, New York

The basic premise of the immune system is simple: to coordinate the activities of various cell types in order to provide extended, if not lifelong, protection against disease-causing pathogens. Usually, this system works flawlessly, quashing infections before they can kill their host and sparking immunity to provide protection against future attacks. Sometimes, however, the system fails and infection prevails—and there is, perhaps, no greater example of this than HIV, a pathogen that almost always succeeds in circumventing and manipulating the body’s immune defense to facilitate its own survival.

Immunology is still a relatively young research field, and there is still much to learn about its function, particularly as it relates to specific pathogens. One of the least-understood members of the immune system family is the dendritic cell. Accounting for only about 1% of all immune system cells, dendritic cells are nevertheless vital to both the initiation and control of immune responses.

Immunologist Melissa Pope, who recently joined the Population Council’s Center for Biomedical Research, has spent more than a decade focusing on dendritic cells and their response to infection with immunodeficiency viruses. In parallel with human studies, Dr. Pope uses rhesus macaques and the simian immunodeficiency virus (SIV) to model HIV infection. And in recent years, Dr. Pope and her colleagues have been focusing on the interactions between dendritic cells and SIV—interactions that should result in a tangible immune response to the virus but instead facilitate infection and dissemination throughout the body. It is hoped that this work will ultimately allow for the identification of ways to successfully block transmission of HIV with microbicidal agents and, quite possibly, provide groundbreaking clues in the continued search for an effective HIV vaccine.

Dendritic Cells 101

As summarized by Dennis Blakeslee, PhD, in an online review of dendritic cells published by the American Medical Association, dendritic cells were discovered in the early 1970s, when immunologists examining the intricate structure of the lymph nodes came across an unusual population of lymphoid cells (Blakeslee, 1998). The cells were large but strangely shaped. Some projected long tendrils between and around neighboring cells, while others had numerous tentacle-like extensions branching out from their membranes. Because these membrane projections looked similar to dendrites on neurons, the cells went on to become known as dendritic cells.

Soon after their discovery, dendritic cells were found almost everywhere in the body. They were documented in virtually all lymphoid tissues, in peripheral blood, in lymphatic fluid, and in numerous nonlymphoid tissues. Because of their widespread distribution, researchers speculated that these dendritic cells could be antigen-presenting cells, which turned out to be the case.

Dendritic cells arise in the bone marrow and, in their immature stage, migrate throughout the body, including the skin and the mucous membranes (e.g., Langerhans cells). Given their wide distribution, they are ideally suited to be the first cells to encounter invading pathogens. Once an encounter is made, the dendritic cells engulf the pathogen(s) and degrade them into protein fragments.

As they migrate via the afferent lymphatic system to the draining lymph tissues, immature dendritic cells evolve into mature dendritic cells. These mature dendritic cells technically reduce their ability to take up and process antigen—although this may not be the case with HIV, which was suggested by Dr. Pope (see “Recent Work” on page 9)—and begin to display the protein fragments from the degraded pathogens on their cell membranes. From there, T-cells and B-cells capable of recognizing the dendritic cells’ major histocompatibility complex (MHC)-peptide antigenic signals make contact—dendritic cells display both MHC class I and class II molecules on their surfaces—resulting in a cascade of immunologic activity.

What makes dendritic cells particularly good antigen-presenting cells? It could be their large, multitenacled shape, which facilitates a great deal of contact with pathogens and other immune system cells. Another noteworthy feature is the large repertoire of molecules expressed on their membranes. As illustrated in Figure 1, these include at least a half dozen antigen uptake receptors, which play a key role in dendritic cells’ abilities to process antigens and to express the specific pathogen peptides. There are also T-cell adhesion and costimulatory molecules, which help

**FIGURE 1. Some Specializations of Dendritic Cells as Antigen Presenting Cells**

**Antigen Uptake Receptors**

**Maturation Receptors**
- TLR5, TNF-R

**T-cell Adhesion and Costimulatory Molecules**
- CD209, CD8 + MHC clusters

Source: Melissa Pope, PhD
Dendritic cells bind with T-cells and activate them once contact has been made. (One molecule in particular, dendritic cell-specific ICAM-3 grabbing non-integrin [DC-SIGN], has been the focus of much research in recent years.) Also present are maturation receptors, which receive signals—in the forms of cytokines like tumor necrosis factor-alpha—to promote their activation or maturation.

In addition to the receptors used for particle uptake, dendritic cells also have another pathway, an exogenous pathway, which can take up dead cells or debris from dying cells, as well as immune-complexed particles—particles bound by or coated with antibodies. "This can be very important in the case of hiv, where you often have immune-complexed virus floating around the body in infected individuals," Dr. Pope explained.

Dendritic cells can be divided into one of two subsets: myeloid dendritic cells and plasmacytoid dendritic cells. Myeloid dendritic cells can be distinguished by the expression of the CD11c molecule. They also express certain toll-like receptors—these are primarily responsible for bar-coding invading microorganisms—mainly TLRs 2, 3, and 4. Myeloid dendritic cells are distributed widely throughout the body and can be found in the blood, lymph, lymphoid tissues, and most notably within mucosal linings of the reproductive and gastrointestinal tracts—prime locations for the transmission of hiv during sexual activity and childbirth/breast-feeding," added Dr. Pope. These dendritic cells respond to GM-CSF, IL-4, and the interferons.

Plasmacytoid dendritic cells express CD123 and TLR9. They are less widely distributed in the body and are usually found in the blood and lymphoid tissues. They express the IL-3 receptor, rendering them sensitive to IL-3, and are unique in their ability to produce enormous amounts of type I interferon, especially in response to a viral infection. "We know that type I interferon can influence the differentiation and activation of myeloid dendritic cells," Dr. Pope said. "So, just by these cells responding to a viral infection, there may be a lot of early cross-talk between the dendritic cell subsets."

**A Trojan Horse**

**As capable as dendritic cells are of kicking off a multipronged immune response to any invading pathogen that crosses their paths, something goes terribly wrong when they are confronted with hiv. "Here lies the paradox," Dr. Pope commented. "The very cells that should be activating the immune system against this pathogen end up facilitating the virus infection."

The mechanisms by which dendritic cells facilitate hiv infection and dissemination throughout the body has been an area of intense focus by various research teams over the past several years. It is known that some dendritic cells can actually become infected with hiv and actively replicate virus. It also appears that the CCR5 receptor on immature plasmacytoid and myeloid dendritic cells permits infection and replication to occur.

Beyond the ability of dendritic cells to become infected with hiv and churn out new viral progeny, other receptors on these cells—including CD4, CXCR4, DC-SIGN, and other C-type lectin receptors—likely play a major role in ferrying the virus to more hospitable CD4+ cells. CD4 is found on most, if not all, dendritic cell subsets, whereas CXCR4 is usually found on mature myeloid and plasmacytoid dendritic cells and DC-SIGN is usually found only on immature myeloid dendritic cells, particularly subepithelial cells in the vaginal lamina propria and rectal mucosal dendritic cells. Other C-type lectin receptors such as langerin on Langerhans cells and the macrophage mannose receptor expressed by several dendritic cell subsets, were also recently shown to play roles in virus capture by specific dendritic-cell subsets (Turville, 2002).

Perhaps the most important factor to consider is the prime location of dendritic cells in the body, particularly those situated within mucosal tissues (see Figure 2). "Mucosal tissues in the vagina and rectum are rich in myeloid dendritic cells, including Langerhans cells in the outer epithelium and sub-mucosal dendritic cells. It’s still not clear if hiv invades the epithelium as a result of trauma, or cell-mediated transfer, or as cell-free virus, but once it’s picked up by the myeloid dendritic cells at the surface, it is then shuttled into draining lymphoid tissue, ultimately resulting in increased virus replication and spread."

**Recent Work**

"To get a better sense of the events that lead to hiv dissemination, Dr. Pope has been involved in a great deal of research looking at the interactions of siv with both immature and mature dendritic cells from humans and rhesus macaques. Most recently, Dr. Pope and her colleagues have been employing a chemically inactivated form of siv—dubbed AT-2 SIV—in studies looking into the virus-dendritic cell biology at the cellular level. ‘We’re talking about whole virus,’ commented Dr. Pope. “It looks and behaves like an infectious virus during the initial cell-virus interplay. All of its surface proteins are still func-
that were exposed to the chemically inactivated virions and their colleagues described studies comparing chemically inactivated simian immunodeficiency viruses (siv) with an infectious organism.

In a recent paper published in the Journal of Virology, Dr. Ines Frank, Dr. Pope, and their colleagues described studies comparing chemically inactivated siv to infectious siv, covering the first 30 to 120 minutes of virus-dendritic cell interplay (Frank, 2002). “We found that whole, chemically inactivated virus was reflective of the interactions of dendritic cells with infectious siv,” Dr. Pope said. “These were important observations, as we can have confidence in the noninfectious virus we’re using.”

Also discussed in this important paper was a side-by-side comparison demonstrating that both immature and mature primate dendritic cells entrap and internalize virus—a first-of-its-kind finding. Initial analyses confirmed that both immature and mature dendritic cells captured considerable amount of fusogenic virus and viral proteins without any apparent impact on the viability, phenotype, or functionality of the cells. Follow-up quantitative reverse-transcription polymerase chain reaction (rt-pcr) analysis indicated that approximately twice as much virus was captured by immature human dendritic cells, compared to their mature counterparts. Conversely, while the distinct cellular distribution of viral proteins and particles was also observed in macaque dendritic cells, the amounts of siv-rna captured in immature and mature macaque dendritic cells were not statistically significantly different and the amount of viral rna associated with each subset was comparable to that of human cells.

Overall, the present estimates of siv-rna genome copy numbers per mature dendritic cell are much greater than those previously reported using semiquantitative dna-pcr techniques, even when smaller amounts of virus were added, which, Dr. Pope purported, “supports the idea that large amounts of virus can be captured by mature dendritic cells but that only a fraction, at best, of this can be subsequently reverse transcribed.” The rt-pcr approach used by Dr. Pope’s group detects all cell-associated virus genomes, not just those that have undergone reverse transcription. Virus simply “entrapped” by the dendritic cells may, of course, remain infectious and contribute significantly to virus spread to cd4+ cells, even in the absence of productive infection of the entrapping dendritic cells. The number of internalized virions—both human and macaque dendritic cells—was significantly higher in mature than in immature cells, and virus that was bound to mature dendritic cells trafficked to a distinct intracellular location compared to immature cells. Most dramatically, large vacuolar compartments that tended to concentrate deeper within the cell containing structurally intact virions were detected only within mature dendritic cells, compared to the peripheral localization of the fewer virions internalized by the immature dendritic cells (see Figure 3).

“We were surprised to find such a large amount of virus in mature dendritic cells,” commented Dr. Pope. “Immature dendritic cells are known to have a greater endocytic capacity than do mature dendritic cells, which down-modulate macropinocytic function as they continue to mature. But here we found that mature dendritic cells were ingesting large numbers of virions.” The reason for this, Dr. Pope explained, can likely be found in the fact that dendritic cells, even as they mature, retain the ability to take up molecules via a number of receptor-mediated mechanisms, including those involving caveolae and clathrin-coated pits—an observation that debunks earlier conclusions that only immature dendritic cells can take up antigens.

Future Directions

Building on these and other study results, additional studies are being conducted to further elucidate the mechanisms of siv (and hiv) binding and internalization in immature versus mature dendritic cell subsets, which will include attempts to define the receptors and uptake systems involved. Also being investigated are the specific intracellular location of internalized virus and the subsequent fate of the virions in different dendritic cell subsets. These studies, Dr. Pope explained, should help clarify the underlying mechanisms of virus retenion and subsequent transmission to t-cells—research that will likely have a significant impact on the development of agents intended to block hiv transmission, such as topical microbicides. By identifying how specific dendritic cell subsets entrap and retain viruses, additional research may pave the way for the development of appropriate strategies that interfere with these processes and impede the early events contributing to the onset of transmission.

It is also hoped that additional studies will yield important insights into how hiv is processed by the different dendritic cells for antigen presentation and immune activation, which has important ramifications for the development of dendritic cell-based vaccines. To this end, understanding how each dendritic cell population handles the virus will allow vaccine developers to identify ways to ensure that these viruses are properly presented to the immune system for the activation of innate and adaptive immunity needed to control or prevent infection.

References

