Dendritic Cells: Immune Activators or Virus Facilitators?

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

SUMMARY BY TIM HORN
EDITED BY MARK DYBUL

T HE BASIC PREMISE OF THE IMMUNE SYS-
tem is simple: to coordinate the activ-
ities of various cell types in order to
provide extended, if not lifelong, pro-
tection 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 circum-
venting and manipulating the body's im-
mune 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. Ac-
counting for only about 1% of all immune
system cells, dendritic cells are neverthe-
less vital to both the initiation and control
of immune responses.

Immunologist Melissa Pope, who re-
cently joined the Population Council's Cen-
ter for Biomedical Research, has spent
more than a decade focusing on dendritic
cells and their response to infection with im-
mune-deficiency viruses. In parallel with
human studies, Dr. Pope uses rhesus
macaques and the simian immunodefi-
ciency virus (SIV) to model HIV infection.
And in recent years, Dr. Pope and her col-
leagues have been focusing on the interac-
tions between dendritic cells and SIV—in-
teractions 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 identifi-
cation of ways to successfully block trans-
mission of HIV with microbicidal agents

and, quite possibly, provide groundbreaking
clues in the continued search for an effec-
tive HIV vaccine.

Dendritic Cells 101

AS SUMMARIZED BY DENNIS BLAKESLEE, PhD,
in an online review of dendritic cells pub-
lished by the American Medical Associa-
tion, dendritic cells were discovered in the
early 1970s, when immunologists exam-
ining 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 nu-
merous tentacle-like extensions branch-
ing out from their membranes. Because
these membrane projections looked simi-
lar 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 lym-
phoid tissues, in peripheral blood, in lym-
phatic fluid, and in numerous nonlymphoid
tissues. Because of their widespread dis-
tribution, researchers speculated that these

dendritic cells could be antigen-presenting
cells, which turned out to be the case.

Dendritic cells arise in the bone mar-
row and, in their immature stage, migrate
throughout the body, including the skin
and the mucous membranes (e.g., Langer-
hans 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 lym-
phatic system to the draining lymph tis-
sues, immature dendritic cells evolve into
mature dendritic cells. These mature den-
dritic 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 pro-
tein fragments from the degraded
pathogens on their cell membranes. From
there, T-cells and B-cells capable of recog-
nizing the dendritic cells' major histocom-
patability complex (MHC)-peptide antigenic
signals make contact—dendritic cells dis-
play both MHC class I and class II mole-
cules on their surfaces—resulting in a cas-
cade of immunologic activity.

What makes dendritic cells particularly
good antigen-presenting cells? It could be
their large, multitentacled 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 anti-
gens 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
CD205, CD206, CD207, BDCA-2, CD209,
ASGP-R, FCγ-R, HSP-R, $\alpha_v\beta5$

Maturation Receptors
TLRS, TNF–RS

T-cell Adhesion and Costimulatory Molecules
CD209, CD8 + MHC clusters
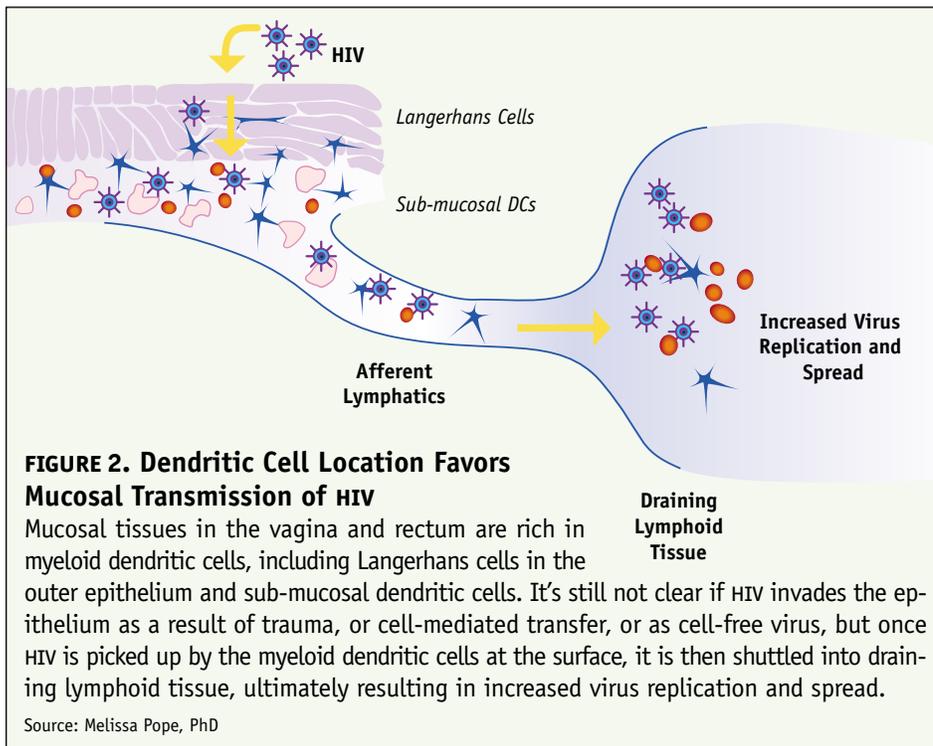
Source: Melissa Pope, PhD

FIGURE 2. Dendritic Cell Location Favors Mucosal Transmission of HIV

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 HIV is 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.

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-
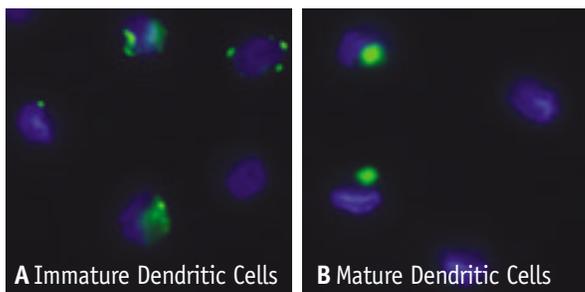
A Immature Dendritic Cells **B** Mature Dendritic Cells

FIGURE 3. **Human Monocyte-Derived Dendritic Cells Exposed to AT-2 SIV**

Human monocyte-derived DCs (immature and mature) that were exposed to the chemically inactivated AT-2 SIV for one to two hours at 37 degrees celsius. The cells were then stained for virus envelope (green) and the nuclei stained with Dapi (blue). Large vacuolar compartments that tended to concentrate deeper within the cell containing structurally intact virions were detected only within mature dendritic cells (B), compared to the peripheral localization of the fewer virions internalized by the immature dendritic cells (A).

Source: Melissa Pope, PhD, Ines Frank, PhD, and Jay Santos.

tioning and are able to bind to specific receptors of the target cells. The difference is that it is not infectious, which is good news in terms of not having to work 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 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 cap-

tured 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—in 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 RE-sults, 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 retention 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. prn

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