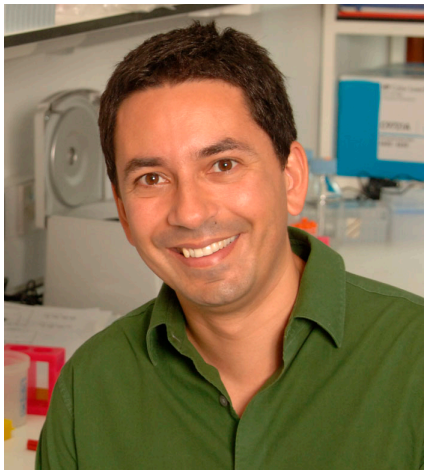
Caetano Reis e Sousa: harnessing DC power

Reis e Sousa is unraveling the pathways that regulate dendritic cell (DC) function in the hope of designing better vaccines and immunotherapies.



Caetano Reis e Sousa

DCs can either magnify or mute immunity by sampling antigens throughout body and presenting them to lymphocytes in combination with secondary signals that either activate or tolerize the responding cell. This quality, which makes DCs a handy weapon against both cancer and autoimmune disease, spurred Caetano Reis e Sousa to dissect the pathways that control different DC functions.

Reis e Sousa first encountered DCs as a graduate student in Jonathan Austyn's laboratory at Oxford University. At the time, these cells were thought to lack the ability to phagocytose antigens—a troubling deficiency in cells known as the initiators of immune responses to microbes. Using DCs found in the skin, Reis e Sousa showed that the inability to pick up antigen was limited to mature cells; freshly isolated ("immature") DCs could engulf particulate antigens with ease (1).

Reis e Sousa's group recently discovered a marker for a DC subset adept at cross-presentation, which might help bring DCs further into the realm of translational medicine.

Determined that his DC expertise become more than skin-deep, Reis e Sousa accepted a post-doctoral position with Ron Germain at the NIH, where he studied antigen processing and cross-presentation by DCs and other cells (2). While at the NIH, he also collaborated with Alan Sher to investigate how microbe-activated DCs induce a T helper (Th)-1 cell response (3).

Since starting his own laboratory at the London Research Institute in 1998, Reis e Sousa has defined several pathways of DC activation that involve Toll-like receptors (TLRs) and non-TLRs such as RIG-I and C-type lectins (4–7). His group recently discovered a marker for a DC subset adept at cross-presentation, which might help bring DCs further into the realm of translational medicine (8).

PATH TO DCS

When did you get interested in science?

I liked biology during my school years in Portugal, but never really got interested in it as a career until I went to high school at the United World College of the Atlantic in Wales. Like many people, I was beginning to get a little philosophical at that age—wondering about the meaning of life and our place in the universe—that sort of thing. I thought biology would offer good answers to these questions. Having said that, my first thought was to study medicine, not biology.

What stopped you?

My academic counselor convinced me that medical school was not for me because I had misspelled "medicine" on the application!

A subliminal message! So how did you end up studying DCs?

After I finished my undergraduate degree in London, I got a government-sponsored scholarship from Portugal that let me stay in the UK to do a PhD. I got accepted into a laboratory at Oxford, but it turned out to be the wrong choice. After three months there, I started desperately going around to every laboratory to see if I could switch, but everyone I talked to was suspicious of why I wanted to move. Fortunately for me, Jon Austyn was one person who didn't have that attitude, and I happily started working with DCs in his laboratory.

A MATURING CAREER

Your thesis work showed the phagocytic nature of immature DCs. What was the impact of that finding?

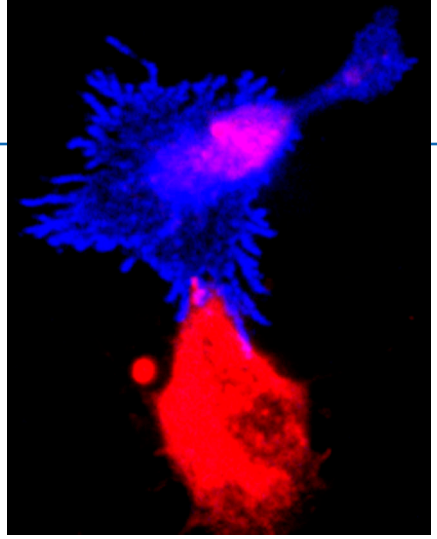
I think it reinforced the notion that DCs have an antigen acquisition stage followed by an antigen presentation stage. Our results from my work with Langerhans cells—a DC subset found in the skin—came out in 1993 in the same issue of the *JEM* in which Ralph Steinman and Kayo Inaba published similar results using DCs from bone marrow progenitors.

What were your post-doctoral goals?

I particularly enjoyed the cell biological aspect of immunology—the antigen processing part. So I went to the NIH to work with Ron Germain, who is an expert on everything, including antigen processing, and who was then interested in working with Langerhans cells. But after spending my entire graduate career peeling ear sheets from hundreds of mice and separating the dermis from the epidermis to isolate these cells, I was bored to tears and really didn't want to touch them ever again.

Did Ron let you off the hook?

Yes, I made a deal with him that I would come to his laboratory to work on anything but Langerhans cells. Ron, being the extraordinary immunologist that he is, gave me the freedom to work on many different things. I first tried to understand how exogenous particulate antigens get presented via the class I MHC pathway. The rule is that this pathway presents endogenous antigens, whereas the exogenous antigens get processed via the class II MHC pathway. But we knew of instances where this rule is broken, and so I studied antigen processing in macrophages to understand the cross-presentation phenomenon.



A CD8α⁺ DC (blue) binds a dying, virus-infected cell (red) and presents viral antigen to killer T cells.

Text and Interview by Hema Bashyam
hbashyam@rockefeller.edu

NEW CONNECTIONS

How did your collaboration with Alan Sher come about?

Alan's group at the NIH was studying how Th1 responses are initiated. Everyone agreed that the cytokine IL-12 was essential, but many argued that only macrophages produced IL-12 in response to microbes, whereas DCs did not. I'd already encountered this sort of argument in my graduate days when we'd disproved the idea that DCs couldn't phagocytose microbial antigens.

I remember betting with Alan that immature DCs that encountered pathogens could produce IL-12. I was proved right when Alan injected mice with a prep of *Toxoplasma* and stained tissues for IL-12: all the cells that stained for cytokine turned out to be DCs. When we published this in 1997, it was effectively the first demonstration that DCs can respond to microbial stimuli by producing IL-12—an idea that is well established today.

Did you continue to work on the DC/IL-12 story when you started your laboratory?

Yes. Others had found that DCs could also make IL-12 in response to CD40 ligation, which made me wonder if these were independent mechanisms or if they somehow worked sequentially. We found that CD40 amplified the effects of the *Toxoplasma* extract. This finding suggested that a microbe interacts with a DC and sets it up for making a certain cytokine profile, and then the CD40 ligand comes along as a neutral stimulus and amplifies the predetermined pattern.

To prove this idea, we went through all of the microbial stimuli that we could get our hands on and looked at their ability to induce two cytokines—IL-12 and IL-10—that were quite important at the time. We identified some stimuli that biased DCs toward IL-12 production and others such as yeast particles that triggered a lot of IL-10 secretion. This was when pattern recognition receptors such as TLRs were becoming interesting, but we found that the IL-10 response to yeast was not TLR-dependent. That's what led us to define a TLR-independent pathway involving C-type lectin receptors.

Did you also continue to work on antigen cross-presentation through the years?

Yes. I began to think that the types of T cell responses one might want to get against tumors are exactly the responses one develops against viruses, and so I tried to understand how DCs sense viruses. We found that they use TLR7 and RIG-I to detect single-stranded RNA viruses. At the same time, we started trying to use DC's cross-priming ability to activate anti-tumor T cells via the class I pathway. CD8α⁺ DCs are particularly good at cross-presenting viral antigens and we found that they can sense virus-infected cells via TLR3. So we thought that loading these DCs with tumor antigens while activating them with TLR3 agonists and anti-CD40 would be an optimal strategy against cancer.

To specifically target these DCs, we borrowed from the work of Ralph Steinman and Michel Nussenzweig, who advocate the use of antibodies to target DCs. We started to characterize molecules that would serve as tags and came up with DNGR1—a C-type lectin receptor—as a possible target on CD8α⁺ DCs. We've shown that immunizing mice with anti-DNGR1 antibodies carrying tumor epitopes and an adjuvant leads to the destruction of melanoma tumors.

Have you found the receptor's natural ligand?

Not yet. We're now exploring its ability to respond to endogenous stimuli, which might explain circumstances in which immunity appears to take place in the absence of microbes. We are also trying to find agonists for other C-type lectins because these receptors might be possible alternatives to TLRs in triggering immunity.

You wrote an essay recently, in which you stressed the importance of terminology in DC biology. What was the reason for this emphasis?

I think language illuminates thinking. And the way in which we employ terminology shapes the way we think. I realized this years ago when I gave a talk at my institute on TLR ligands triggering this and that. One of my colleagues, a structural biologist, asked me about the ligands' affinity for their receptors. I had to confess that we had no real biochemical proof that they were ligands because all we were doing was treating the cells with compound X and seeing whether it induced a response or not. And in fact, the correct pharmacological term that I should have used was "agonist," because a ligand can also be an antagonist. Another case in point: no two immunologists use the word "tolerance" to mean the same thing! I work in an institute that's mostly nonimmunological, and I realize that many of my colleagues dislike immunologists, with good reason sometimes, because we are very wishy-washy with terminology.

"I think language illuminates thinking. And the way in which we employ terminology shapes the way we think."

1. Reis e Sousa, C., et al. 1993. *J. Exp. Med.* 178:509–519.
2. Reis e Sousa, C., and R.N. Germain. 1995. *J. Exp. Med.* 182:841–851.
3. Reis e Sousa, C., et al. 1997. *J. Exp. Med.* 186:1819–1829.
4. Rogers, N.C., et al. 2005. *Immunity.* 22:507–517.
5. LeibundGut-Landmann, S., et al. 2007. *Nat Immunol.* 8:630-638.
6. Schulz, O., et al. 2005. *Nature.* 433:887–892.
7. Pichlmair, A., O. 2006. *Science* 314:997-1001.
8. Sancho, D., et al. 2008. *J. Clin. Invest.* 118:2098–2110.