Immunology

Inside the gearbox of the dendritic cell

Colin Watts

Dendritic cells are a group of 'professional' antigen-presenting cells that seem to hold the key to the induction of T-cell responses. For this reason, there is a great deal of interest in their fundamental properties and how they might be used for immunotherapy — for example, to stimulate immune responses against tumours or infectious agents. Two papers by Cella *et al.*¹ and Pierre *et al.*² (pages 782 and 787 of this issue), reveal how environmental factors can trigger dendritic cells to reorganize and redirect their antigen-processing apparatus, to maximize their antigen-presenting and, hence, T-cell triggering capacity.

T lymphocytes are activated upon recognition of cell-surface major histocompatibility complex (MHC) class I and class II molecules carrying specific, processed, antigenic peptides. Although MHC molecules are expressed by many different cell types, only professional antigen-presenting cells and, in particular, dendritic cells, can trigger naive T cells³. Once over this hurdle, however, activated T cells can engage other cells expressing the same specific peptide–MHC class I or class II complexes.

The new studies^{1,2} are not the first to

report that dendritic cells modulate their antigen-presenting capacity upon maturation. Earlier work^{4–7} showed that, when these rare cells were isolated from tissues such as skin or spleen, a terminally differentiated population was obtained. These cells expressed high levels of MHC molecules on the cell surface, but had low rates of new MHC biosynthesis. As a result, they stimulated T cells that could recognize the existing surface MHC–peptide complexes (generated either *in situ*, or soon after tissue disruption), but they were poor at capturing and presenting newly offered antigens.

Later studies showed that this switch from an antigen-capturing mode to an antigen-presenting mode — could be induced in cultured dendritic cells by cytokines such as tumour-necrosis factor- α , and bacterial products such as lipopolysaccharide^{8,9}. These agents also induced the dendritic cells to migrate, *in vivo*, from non-lymphoid tissues to the T-cell-rich areas of lymphoid organs^{10,11}. So, the life history of the dendritic cell is played out in two parts. First it acts as a 'sentinel', sampling and processing the local milieu. Later, following migration, it

becomes a reporter of its earlier environment, which is expressed in peptide form on class II (and probably class I) MHC molecules. At this mature stage there are also elevated levels of adhesion molecules and 'co-stimulators' of T-cell triggering.

Although there are clear echoes of the earlier studies, Cella *et al.*¹ and Pierre *et al.*² have now reconstituted the distinct stages of the dendritic-cell life history under controlled conditions *in vitro*. They also provide new insights into the organization of class II MHC maturation, expression and stability at each of these stages. Both studies exploit methods for generating immature dendritic cells *in vitro*, either from blood monocytes using granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (ref. 8), or from proliferating bone-marrow precursors¹² using GM-CSF.

For successful expression of antigenic peptide–MHC class II complexes on the cell surface, the endocytosed antigen needs to be efficiently mixed with newly synthesized class II MHC molecules, in specialized compartments of the endosome/lysosome system (reviewed in ref. 13). Proteases and specific regulators of peptide loading are also required. The assembled complexes must then be transported to the cell surface, where they should ideally be long-lived to maximize the opportunity for interaction with T cells. The work of Cella, Pierre and their colleagues now shows that dendritic cells can modulate these parameters to control antigen presentation.

Figure 1 Dendritic cells hold the key to T-cell responses — they present antigenic peptides complexed with class II major histocompatibility complex (MHC) molecules to T cells. Cella *et al.*¹ and Pierre *et al.*² have used human and mouse dendritic cells, respectively, to work out how this is regulated as dendritic cells mature. In response to environmental factors (especially those that are normally associated with pathogenic invasions), dendritic cells reorganize themselves, to maximize their ability to present antigen. In an 'idling' immature dendritic cell (a), endocytosis (red squares) and MHC biosynthesis occur, but the peptide–MHC class II complexes (red ball and stick) accumulate in lysosomes (mouse), or reach the cell surface but are then recycled (human). In response to bacterial products (such as lipopolysaccharide, LPS; cytokines such as tumour-necrosis factor- α , TNF- α ; or as-yet undefined factors), the dendritic cells change gear and start to mature (b). There is increased biosynthesis of class II MHC, which maximizes specific loading of antigen (green squares). Peptide loading may occur in diverse compartments, including CIIV^{2,14} and recycling endosomes¹. In the mature dendritic cell (c) the peptide–MHC class II complexes are expressed efficiently on the cell surface (green ball and stick), where they have a dramatically extended half-life because endocytosis is shut down. Increased levels of adhesion and co-stimulatory molecules (yellow) complete the surface phenotype that is needed to trigger naive T cells. Antigen that is captured in the idling and/or maturing state can therefore be very efficiently presented to T cells in the maturing state. P, compartments of peptide loading; E, recycling endosome; L, lysosome; C, CIIV-like endosome.

A key finding in both studies is that, although immature dendritic cells are active in the biosynthesis of class II MHC and in peptide loading, the assembled peptide-MHC class II complexes are not stably expressed on the cell surface. Instead, they accumulate in lysosomes² or, in the human-monocyte-derived dendritic cells¹, the complexes reach the cell surface, but they are removed and become degraded relatively quickly. It is as though the machinery for antigen processing is running but, at this stage, the cell is set up in an 'idling' mode and has not found the right gear to drive the effective presentation of antigen to T cells. As shown in Fig. 1, the idling state differs between the two systems in terms of class II MHC routing and steady-state distribution.

Maturation — induced by microbial products or cytokines in the human cells, and by as-yet unidentified factors in the murine cultures used by Pierre *et al.*² — engages the correct gear to drive the expression of class II MHC molecules onto the cell surface. Maturation also transiently accelerates class I and class II MHC biosynthesis, particularly in the human cells. In the dendritic cells from murine bone marrow, the class II MHC is redistributed from lysosomes in the immature cells, to peripherally located endocytic vesicles. These resemble a specialized class II MHC-rich population (CIIV), previously found in B cells¹⁴, which are constitutively active antigen-presenting cells. Finally, fully mature cells in both systems express almost all of their class II MHC on the cell surface (Fig. 1).

How much of the class II MHC in the CIIV-like endosomes is salvaged from the earlier lysosome pool, and how much arises from new biosynthesis? Although Pierre *et al.* do not directly address this, newly assembled class II MHC molecules are now clearly directed to the cell surface, instead of to lysosomes. Importantly, the sequential changes in the distribution of class II MHC molecules seen in the murine bone marrow cultures are also observed *in vivo* in murine Langerhans cells migrating out of, or maturing within, explanted skin².

Both groups find that antigen captured by immature cells in an early time window can be presented by the same cells at a later stage, providing their maturation programme begins soon after antigen capture. Most peptide-MHC class II interactions are irreversible, so 'memory' of earlier antigenic encounters depends on the half-life of cell-surface class II MHC (ref. 15). This is increased up to ten-fold on mature, compared with immature, dendritic cells^{1,2}. A long half-life is important *in vivo*, as the immature cell migrates from the periphery — where it has captured antigen — to the lymphoid organs, where its antigenic load is scanned by T cells. The increased half-life is due to reduced endocytosis of class II MHC

(ref. 1), although it is not known whether this occurs through down-regulation of macropinocytosis¹⁶ or clathrin-mediated endocytosis, or both.

Cella *et al.*¹ also find that antigen offered during the transient boost in class II MHC biosynthesis (which is triggered, in this case, by tumour-necrosis factor- α) is loaded on class II MHC to a higher 'specific activity' than before. This means that microbial products 'help' to eliminate the pathogen, by stimulating the preferential presentation of microbial antigens. They may do this directly, through lipopolysaccharide, or indirectly through cytokines such as tumour-necrosis factor- α .

We now need to understand the molecular basis of the changes in class II MHC trafficking which characterize the maturation of dendritic cells. For example, why do class II MHC molecules accumulate in lysosomes in immature murine dendritic cells? At what level is this controlled? What drives the constitutive membrane ruffling and endocytosis in immature dendritic cells¹⁶, and how is this shut off in mature cells? The search for gene products that are differentially expressed in mature versus immature dendritic cells is already under way. Some may be relevant to these changes.

Finally, these studies may be relevant to the use of dendritic cells for clinical immunotherapy. The hope is that dendritic cells — obtained *in vitro* from patients' blood — could be loaded with known or putative tumour antigens, and then used as immunogens to elicit anti-tumour cytotoxic T-cell responses. Studies in animal models have already shown the potential of this approach (reviewed in ref. 17). Related strategies, such as direct fusion of tumour cells with dendritic cells, have also been described (see the News and Views article by Hart and Colaco on pages 626–627 of last week's issue).

Tumour-antigen-feeding protocols designed to generate specific class I and class II MHC T-cell determinants on cultured dendritic cells could take advantage of both the transient boost in MHC biosynthesis which is seen at the outset of maturation, and the dramatically prolonged half-life of class II helper determinants on fully mature cells. □

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Daedalus

Magnetic autoeroticism

Last week Daedalus presented a magnetic nerve stimulator. It launched magnetic pulses into the spinal cord or other nerve pathways. Its pulses could be tailored in shape and frequency to induce firing currents only in specific nerves — say, those to a certain muscle. The user could thus control that muscle more precisely.

He is now applying the technology to muscles we cannot control at all. A magnetic heart pacemaker, for example, need not be implanted. It could be strapped on the chest, from where it could be adjusted at will. If the wearer wished to run for a bus, he could speed up his heart for the extra effort. Similarly, the widespread miseries of constipation could be cured by a magnetic belt, tuned to the key parasympathetic nerves in the spinal cord. The wearer could defecate on command, simply by pressing a button.

The most widespread miseries of them all, muses Daedalus, are probably sexual. A magnetic belt tuned to the appropriate spinal nerves could induce sexual arousal and even orgasm on command. It would transform the lives of those trapped in unhappy or fading relationships. But too much sex is probably as troublesome as too little. Thus, rape, murder, robbery and pop-music composition are all forms of sexual behaviour: they are overwhelmingly the work of young, sexually driven males. Such offenders are already being marked by special electronic tags. Daedalus proposes a 'neuromagnetic tag', again in spinal belt form, to control the wearer's sexuality. The obvious idea is to block it completely. But Daedalus favours the converse approach. His neuromagnetic tag will stimulate the nerves of orgasm as frequently and intensely as possible. This will exhaust the wearer's sexual drive. Without any other stimulation, he will find himself having repeated involuntary orgasms as fast as his physiology can manage it. A rapist or sex offender would be permanently drained, 'shorted out' and made harmless by such a tag. And if sex underlies the rest of the portfolio of male behaviour, then murder, robbery, pop-music composition and other male social nuisances would be curtailed too. Their exponents could safely be released into the community. At intervals they would show a certain sudden inwardness and self-absorption, but would otherwise be quiet, undisturbed citizens. The treatment could not even be condemned as a cruel and unusual punishment. It would be a pleasurable and unusual punishment.

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