



Lincoln's Inn Fields

Immunobiology

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Priming and directing T cell responses is the principal function of dendritic cells (DC), the major class of antigen-presenting cells (APC) in the body. DC are a heterogeneous family of leucocytes present as a trace population in most tissues, including solid organs, skin and mucosa.

From these tissues DC migrate into lymph nodes, spleen and other secondary lymphoid organs carrying antigens captured at the site of origin. Similarly, DC ordinarily resident in secondary lymphoid organs capture antigens that reach those tissues via the lymph or blood.

Depending on context, the presentation of antigens by DC can lead to T cell expansion and productive immune responses but can also inactivate T cells or promote the development of suppressor cells that maintain tolerance [Steinman *et al.*, (2002). *Proc. Natl. Acad. Sci. U.S.A.* 99:351-358]. Therefore, the specific targetting of antigens to DC allows for numerous possibilities of immune manipulation, ranging from the induction of tolerance to improved vaccination against infectious diseases and cancer:

DNGR-I

To target antigens specifically to DC one needs to identify cell surface markers restricted to those cells. Such markers should act as endocytic receptors that will deliver bound cargo to compartments where antigen processing for MHC class I and class II presentation can take place. Several years ago, we had identified a fragment of a C-type lectin receptor in a screen designed to find transcripts selectively expressed in a subset of DC called CD8 α^+ DC. The full cDNA was

subsequently cloned and turned out to correspond to the *dec9a* gene, part of the NK lectin gene family that also includes Dectin-1, another C-type lectin of interest to the lab. We subsequently confirmed the DC-restricted expression pattern of *dec9a* in mouse and human and, accordingly, named the protein product 'Dendritic cell NK lectin Group Receptor 1' (DNGR-1) [Sancho *et al.*, (2008) *J Clin Invest* 118:2098-2110].

We made monoclonal antibodies to mouse and human DNGR-1 and used them to identify the cell populations that express the receptor. In the mouse, DNGR-1 is expressed at high levels by CD8 α^+ DC and at low levels by plasmacytoid DC (pDC) but not by other mouse hematopoietic cells [Sancho *et al.*, (2008) *J Clin Invest* 118:2098-2110]. DNGR-1 is expressed at the plasma membrane but possesses endocytic activity and internalises bound antibody [Sancho *et al.*, (2008) *J Clin Invest* 118:2098-2110]. This fact, together with its selective expression pattern, suggested that DNGR-1 might be useful for antigen targeting to DC, much like another C-type lectin known as DEC-205 [Hawiger *et al.*, (2001) *J. Exp. Med.* 194:769-779, Bonifaz *et al.*, (2002) *J. Exp. Med.* 196:1627-1638]. We went on to show that fluorescently-labelled anti-DNGR-1 mAbs injected into mice selectively label CD8 α^+ DC (strongly) and pDC (weakly) and that a derivative of the OVA 257-264 H-2K^b-restricted peptide covalently coupled to anti-mouse DNGR-1 is selectively crosspresented on MHC class I by CD8 α^+ DC *in vivo* [Sancho *et al.*, (2008) *J Clin Invest* 118:2098-2110]. When given with adjuvant, the same conjugates induce potent OVA-specific cytotoxic T cell responses, which can prevent development, as well as mediate eradication, of OVA-expressing B16 melanoma [Sancho *et al.*, (2008) *J Clin Invest* 118:2098-2110]. However, to make the approach more relevant to cancer therapy, we also determined whether immunity could be achieved against relevant tumor antigens. This was achieved by conjugating anti-DNGR-1 to

peptides corresponding to endogenous melanocyte differentiation antigens that can act as B16 tumor rejection antigens in H-2^b mice. Notably, when given therapeutically, a single dose of 2 μg of conjugates (equivalent to 50ng of peptides), together with adjuvant, is able to break self-tolerance and induce crosspriming of CTL, which mediate eradication of growing tumour [Sancho *et al.*, (2008) *J Clin Invest* 118:2098-2110]. Thus, targeting antigens to DNGR-1 is a promising approach for therapeutic vaccination, at least in the mouse. An exciting development is that we and others have found that human DNGR-1 is also highly restricted in expression to a small subset of blood DCs [Sancho *et al.*, (2008) *J Clin Invest* 118:2098-2110, Caminschi *et al.*, (2008) *Blood* 112:3264-3273, Huysamen *et al.*, (2008) *J. Biol. Chem.* 283:16693-16701]. This suggests it might also be useful for selective DC targeting in humans and sets it apart from DEC-205, which, in human, has a broad distribution and is unsuitable as a target [Kato *et al.*, (2006) *Int Immunol* 18:857-869]. We have filed a patent application on DNGR-1 as a novel and highly specific marker of mouse and human DC subsets that can be exploited for antigen delivery and for CTL crosspriming and tumor therapy.

Dectin-1

In order to ensure that the response to antigen culminates in immunity, antigen targeting to DC needs to be combined with additional strategies to 'activate' the cells [Reis e Sousa, C. (2006) *Nat Rev Immunol* 6:476-483]. Dectin-1, a phagocytic receptor for β-glucans expressed on yeast and bacterial cell walls (Figure 1), can function as a DC activating receptor [Rogers *et al.*, (2005) *Immunity* 22:507-517]. Over the last year, we and others have shown that cell activation requires sustained signalling via Dectin-1, which takes place at the cell surface before ligand internalisation and is therefore especially prominent in response to ligands that are too large to be phagocytosed [Rosas *et al.*, (2008) *J. Immunol.* 181:3549-3557, Hernanz-Falcón *et al.*, (2009) *Eur. J. Immunol.* 29:507-513]. We further showed that Dectin-1-activated DC can induce the conversion of regulatory T cells into IL-17 producers [Osorio *et al.*, (2008) *Eur. J. Immunol.* 38:3274-3281]. The latter observation extends our earlier studies on the induction of immunity dominated by IL-17-producing

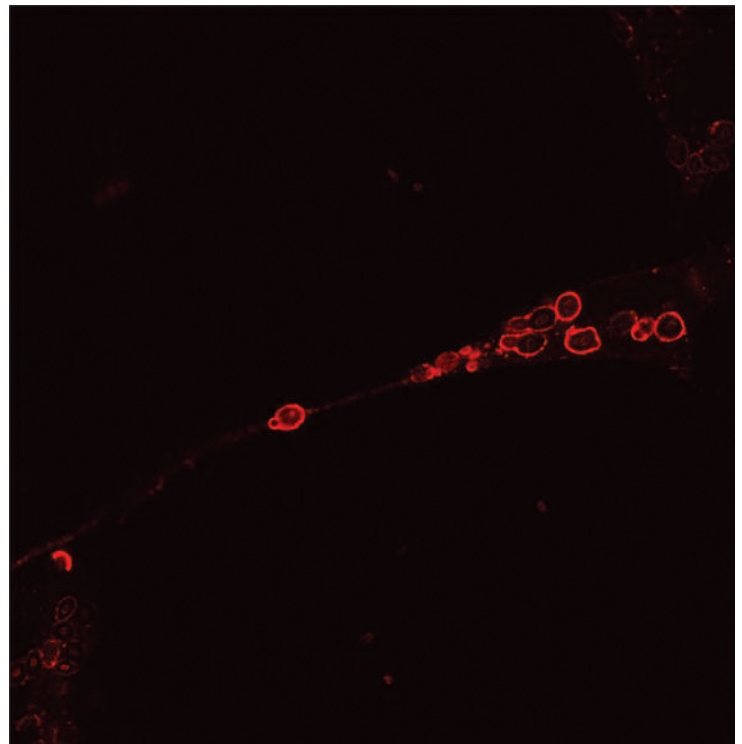


Figure 1. Cherry-Dectin-1-expressing HEK293 cells were allowed to internalise zymosan, a yeast cell preparation, for 1h before imaging by confocal microscopy. Note the enrichment for Dectin-1 at zymosan-containing phagosomes and phagocytic cups.

cells upon Dectin-1 triggering *in vivo* [Leibundgut-Landmann *et al.*, (2007) *Nat Immunol* 8:630-638]. In addition, we have also shown that DC presenting OVA and stimulated with the Dectin-1-specific agonist, curdlan, are competent to prime OT-I TCR transgenic T cells *in vitro* and that curdlan serves as a potent adjuvant *in vivo* for the induction of OVA-specific cytotoxic T cells. The latter can protect mice from OVA-expressing B16 melanoma [Leibundgut-Landmann *et al.*, (2008) *Blood* 112:4971-4980]. These studies indicate that the Dectin-1 pathway can also regulate CD8⁺ T cell immunity and could therefore be exploited in the design of cancer vaccines.

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