Found in translation: the human equivalent of mouse CD8$^+$ dendritic cells

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The murine dendritic cell network comprises multiple subsets with distinct functions, but few of their human counterparts have been described. New data now reveals the likely human equivalent of the mouse DC subset specialized in cross-presentation.

Often described as a network of largely homogeneous cells distributed throughout the body, the dendritic cell (DC) system is, in fact, composed of distinct subtypes which, like the pieces of a puzzle, come in distinct shapes and sizes. Medical research laboratories worldwide have enthusiastically embraced the characterization of these subsets using mice as the basic experimental model. One result has been a wealth of information on the specialized roles of different subtypes of murine DC in tolerance and immunity (Heath and Carbone, 2009). But how much of this detailed information is applicable to the human immune system? Until recently, the clinical relevance of the various DC subsets had not been apparent. The subtleties of the murine DC system seemed "Lost in Translation" (Merrill, 1974). Four papers in this issue now make progress toward resolving this problem in identifying the human counterpart to the mouse cross-presentation specialists, CD8$^+$ DCs (Bachem et al., 2010; Crozat et al., 2010; Jongbloed et al., 2010; Poulin et al., 2010).

What are we looking for?

Some aspects of the human and mouse DC systems already appeared to be well aligned. The major division into plasmacytoid and conventional DCs, for example, is accepted for both species. Both species also have at least one subset of migratory, conventional DCs in the dermis and a separate subset, known as Langerhans cells, in the epidermis. A notable discrepancy between the two systems has been at the level of the resident DC populations in lymphoid tissues. In the mouse, two functionally distinct populations have been recognized, one with high surface expression of CD8α, the other lacking this marker. No human DC expressing CD8 had been observed. To quote Merrill (1974): "Lost, is it, buried? One more missing piece? But nothing's lost. Or else: all is translation, And every bit of us lost in it."

As it turns out, however, CD8 is a poor marker of the eponymous mouse DC subset, as this molecule has no known role in DC development or function (for review see Shortman and Heath, 2010). Furthermore, an immediate precursor of CD8$^+$ DC can be identified in mouse blood and lymphoid tissue, and this precursor expresses all the signatures of this population except for CD8. The absence of CD8 expression in the human DC system is therefore not particularly surprising. Fortunately, CD8$^+$ DCs possess additional features that are not strictly unique to this population, but together provide an accurate description of this subset (Table I). First, many other surface molecules in addition to CD8 are differentially expressed in CD8$^+$ DCs (Segura et al., 2010) and several represent useful markers for subset discrimination. Second, the development of CD8$^+$ DCs is strictly dependent on expression of the transcription factors Batf3 and IRF-8 (Schiavoni et al., 2002; Hildner et al., 2008). CD8$^+$ DCs are also distinct in their expression of TLR3, which is not expressed by CD8$^-$ DCs. However, they express little or no TLR1, TLR6 or other TLR2 coreceptors, and also lack TLR7 and the cytosolic receptor RIG-I (Edwards et al., 2003; Luber et al., 2010; Segura et al., 2010). Fourth, DC subsets differ in the pattern of cytokines they secrete upon activation, and CD8$^+$ DCs stand out as the major producers of interleukin (IL)-12 (Reis e Sousa et al., 1997). Finally, two unique features of CD8$^+$ DCs that have attracted considerable attention in recent years are their ability to capture dead cells and to cross-present different forms of exogenous antigens on their major histocompatability complex (MHC) class I molecules (for review see Villadangos and Schnorrer, 2007).

Where should we be looking?

Armed with this profile, the authors of two of the studies in this issue sought the equivalent of CD8$^+$ DCs in human spleens (Poulin et al., 2010) and tonsils (Jongbloed et al., 2010). Their choice of organs was not fortuitous. In mice, the final development of lymphoid organ–resident DCs occurs within the organs themselves (Shortman and Naik, 2007), so these would be the tissues of choice for purifying CD8$^+$ DCs. In fact, previous studies had suggested the existence of human CD8$^+$ DCs equivalents in thymi and tonsils (Vandenabeele et al., 2001; Galibert et al., 2005). Sure enough, Poulin et al. (2010) found three DC populations in human spleens, only one of which expresses Clec9A (DNGR-1), Necl2, and IRF8, which are typical markers of mouse CD8$^+$ DCs (Table I). Examining human tonsils, Jongbloed et al. (2010) also found Clec9A$^+$ DCs

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localized in the T cell areas, the sites where mouse $CD8^+$ DCs preferentially accumulate at steady state. These were encouraging findings, but fell short of a true characterization of a functional equivalent of mouse $CD8^+$ DCs in humans. The ethical and logistical difficulties inherent to the purification of human lymphoid organ DCs have so far precluded such characterization, so the groups sought human $CD8^+$ DC equivalents from three other potential sources: blood, cultures of cord blood stem cells, and humanized mice.

That human blood contains several types of conventional DCs has been known for some time. MacDonald et al. (2002) defined four subsets, one of which, the CD141 (BDCA3)$^+$ DC, was already known to possess mouse $CD8^+$ DC-like features, including Clec9A expression (Caminschi et al., 2008; Huysamen et al., 2008; Sancho et al., 2008). Furthermore, in a groundbreaking study by Robbins et al. (2008), gene chip (meta)analysis of the transcriptome of multiple mouse and human DC subsets indicated a close relationship between mouse $CD8^+$ DCs and human blood $BDCA3^+$ DCs. Three of the current studies confirmed that this population is likely the human equivalent of mouse $CD8^+$ DCs (Bachem et al., 2010; Crozat et al., 2010; Jongbloed et al., 2010).

Although blood may be a more accessible source of human DCs than lymphoid organs, the low frequency of $CD141^+$ DCs (1 in 10^4 PBMCs) makes the purification of this population a cumbersome and expensive process. An obvious alternative would be to generate these cells in culture from earlier hematopoietic precursors. After all, it was the optimization of culture systems for the generation of DCs from bone marrow and blood precursors that put DC studies within reach of many laboratories and catalyzed the expansion of the field (Inaba et al., 1992; Sallusto and Lanzavecchia, 1994). One limitation of the original granulocyte/macrophage colony-stimulating factor (GM-CSF)–based culture systems is that they generate monocyte-derived DCs (Xu et al., 2007), a subset distinct from all other DC types, including $CD8^+$ DCs (Shortman and Naik, 2007). However, the use of alternative growth factors, especially Flt3 ligand, allows for the generation of a mouse $CD8^+$ DC equivalent whose only discrepancy with $CD8^+$ DCs appears to be the expression of CD8 itself (Naik et al., 2005). Surely, a similar protocol applied to human precursors would produce human DCs resembling $CD8^+$ DCs? Applying this logic, Poulin et al. (2010) obtained $CD141^+Clec9A^+$ cells after culturing cord blood hematopoietic stem cells with medium containing stem cell factor, GM-CSF, IL-4, and Flt3L. These comprised a low proportion of the cells in culture, but were still present at a significantly higher frequency compared with blood.

Humanized mouse spleens represent the fourth source of human $CD141^+$ DCs (Cravens et al., 2005), and this was also used by Poulin et al. (2010) for further characterization of this subset.

Similarities and discrepancies

How similar are mouse $CD8^+$ DCs and human $CD141^+$ DCs? Two of the new studies show that human blood $CD141^+$ DCs express the chemokine receptor XCR1 (Bachem et al., 2010; Crozat et al., 2010), an important finding considering that $CD8^+$ DCs appear to be the only cells in the mouse that express this molecule (Dorner et al., 2009). This makes XCR1 a promising marker for cell identification with one caveat: in these studies, the level of XCR1 was only assessed by PCR even though its differential expression in DC subsets has been confirmed using functional assays (Dorner et al., 2009; Crozat et al., 2010) and membrane proteomics (Segura et al., 2010). This suggests that staining with anti-XCR1 antibodies is not a practical option for DC subset discrimination. Another similarity between $CD141^+$ DCs and $CD8^+$ DCs is that they express the transcription factors Batf3 and IRF-8 (Jongbloed et al., 2010; Poulin et al., 2010) and lack expression of IRF-4, a factor required for development of some mouse DC types, but not $CD8^+$ DCs.

Table I. Defining properties of mouse $CD8^+$ DCs and human $CD141^+$ DCs

Property	Mouse $CD8^+$ DCs	Human $CD141^+$ DCs	Human equivalent references
Surface markers[a]	$CD8^+$, $CD11b^{low}$, $CD24^{hi}$, $CD36^+$ $CD205^+$ $CD172a^-$ $Clec9A^+$ $DCIR2^-$ $Necl2^+$, $XCR1^+$	$CD1^-$ $CD141^+$ $Clec9A^+$ $Necl2^+$ $XCR1^+$	Bachem et al., 2010; Crozat et al., 2010; Jongbloed et al., 2010; Poulin et al., 2010
Developmental transcription factors	$Batf3^+$, IRF-8^+, IRF-4^-	$Batf3^+$, IRF-8^+, IRF-4^-	Jongbloed et al., 2010; Poulin et al., 2010
Pathogen sensors[a]	$TLR1^{-/low}$ $TLR2^+$ $TLR3^+$ $TLR4^+$ $TLR6^{-/low}$ $TLR7^-$ $TLR9^+$ $TLR11/12^+$ RIG^-	$TLR3^+$, $TLR7^-$, $TLR9^-$	Jongbloed et al., 2010; Poulin et al., 2010
IL-12 production	Yes	Yes	Jongbloed et al., 2010; Poulin et al., 2010
Dead cell uptake	Yes	Yes	Jongbloed et al., 2010; Poulin et al., 2010
Antigen cross-presentation	Yes	Yes	Bachem et al., 2010; Crozat et al., 2010; Jongbloed et al., 2010; Poulin et al., 2010

[a]Only markers normally used for $CD8^+$ DC or $CD141^+$ DC subset discrimination are listed. Clec9A is also known as DNGR1. CD172a is also known asSirpα . CD141 is also known as BDCA 3.

$CD141^+$ DCs express TLR3, but not TLR7, and secrete IL-12 when activated through TLR3 (Jongbloed et al., 2010; Poulin et al. 2010). Finally, $CD141^+$ DCs are capable of phagocytosing dead cells and cross-presenting cell-associated and soluble antigens (Jongbloed et al., 2010; Poulin et al., 2010). Based on these criteria, the studies make an excellent case that $CD141^+$ DCs represent a human equivalent of mouse $CD8^+$ DCs (Table I).

Alas, there are some differences between the conclusions of the new studies on human $CD141^+$ DCs and what we know about mouse $CD8^+$ DCs. TLR9 is expressed in $CD8^+$ DCs but not in $CD141^+$ DCs, although this is not surprising, as human conventional DCs in general appear to lack TLR9 (Jongbloed et al., 2010). In the mouse, IL-12 production is tightly controlled and requires two signals, one provided by a TLR ligand, the other by either CD40 engagement or by a combination of cytokines. So far, however, the second signal requirements for IL-12 production appear to be different for human $BDCA3^+$ DCs. Poulin et al. (2010) found that although CD40 engagement was not effective, activated T cells could provide a second signal for IL-12 production. Jongbloed et al. (2010) found that a mix of cytokines could provide a second signal, but the mix appears to be different from the effective mix for the mouse $CD8^+$ DCs.

The specialization of $CD8^+$ DCs in cross-presentation is another feature that does not appear clearly mirrored in $CD141^+$ DCs. One difficulty in assessing this activity is that many cell types, including non-DCs, appear capable of cross-presentation to some degree in vitro (Villadangos and Schnorrer, 2007; Villadangos and Young, 2008). $CD8^+$ DCs are described as specialized in this role, in part because mice lacking $CD8^+$ DCs are defective at cross-presentation (Hildner et al., 2008; Lin et al., 2008), and because $CD8^+$ DCs are far more efficient at delivering antigen into the cross-presentation pathway compared with other DC types under conditions of equivalent antigen uptake (Schnorrer et al., 2006). Monocyte-derived DCs also appear to be efficient cross-presenters, but their contribution is probably restricted to inflammatory conditions (Segura et al., 2009).

To conclude that $CD141^+$ DCs are specialized in cross-presentation in humans, it would be desirable to perform a side-by-side comparison of their capacity to capture and present antigen via MHC class I and II relative to other DC types. Because DC availability is a problem, it is understandable that the studies in this issue lack this type of analysis. Certainly all four studies make strides toward this goal (Bachem et al., 2010; Crozat et al., 2010; Jongbloed et al., 2010; Poulin et al., 2010), but the comparisons are not comprehensive. In one study, $CD141^+$ DCs were slightly more efficient at cross-presentation than were monocyte-derived DCs (Poulin et al., 2010), and in the other studies, $CD141^+$ DCs appeared to cross-present more efficiently than plasmacytoid DCs or conventional $CD1^+$ DCs (Bachem et al., 2010; Crozat et al., 2010; Jongbloed et al., 2010). However, these differences were not as clear-cut as in the mouse system. Furthermore, cross-presentation by the human DCs required activation via TLRs, in apparent contrast to mouse $CD8^+$ DCs. The magnitude of antigen presentation in the human DCs assays was read out by measuring the number of IFN-γ–producing cells elicited by the DCs (priming), so factors other than the efficiency of generation of MHC I–peptide complexes may have influenced the outcome. On the other hand, it must be stressed that the experiments that have revealed different antigen presentation capacities of mouse DC subsets in vivo used cells purified from lymphoid organs (Villadangos and Schnorrer, 2007; Villadangos and Young, 2008), whereas the experiments on human DCs used cells purified from blood or produced in culture. Perhaps the $CD141^+$ DCs are precursors of a population whose cross-presentation potential is only fully realized after they reach terminal differentiation in lymphoid tissue.

What next?

Some non-DC aficionados have understandably felt confused and even irritated by our preoccupation with DC subsets. Was it necessary to add more complexity to the description of the DC network by defining new subpopulations? To quote Merrill's again, "But hidden here is a freak fragment, Of a pattern complex in appearance only."

Confusing as the DC network may appear, the characterization of its components reveals how these interlock to form a coherent picture. We now know much more about one of the hitherto hidden pieces of the human DC puzzle because its mouse counterpart had already been carefully examined. Targeting antigens to mouse $CD8^+$ DCs is an efficient strategy to elicit antitumor immunity (Caminschi et al., 2009), and the question now is whether similar approaches can be developed to harness the human DC counterparts. We anticipate that the characterization of the human DC types that remain hidden will help understand how the whole system works and will open new opportunities to achieve the ultimate translation that we all seek: from basic mouse science to human clinical outcomes (Steinman and Banchereau, 2007).

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