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Commentary

The Hierarchy of Antigen Delivery

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Vaccination is one of the greatest success stories in medicine. Effective vaccines contain adjuvants that activate dendritic cells (DCs) and of course the antigen(s), which are taken up by DCs and, after processing into peptides, are presented on MHC molecules to activate T cells. T cells are the backbone of adaptive immunity. Cytotoxic CD8 T cells kill cells expressing peptides from intracellular microbial or tumour antigens on MHC class I (MHC-I) molecules. CD4 T cells recognise antigens derived from endocytosed antigens presented on surface MHC-II molecules and respond by producing factors that help other immune cells. Arguably the most important helper function is promoting antibody production by B cells, but also CD8 T cells require help from CD4 T cells. Importantly, before performing effector functions, T cells first must be activated by antigen-presenting cells, usually DCs. DCs are crucial for activating CD8 T cells against endocytosed antigens, by diverting them into specialised early endosomal compartments for MHC-I presentation. This so-called cross-presentation is critical for CD8 T cell responses against tumours, viral infections and all vaccinations strategies (Kurts et al., 2010). In the current issue of *EBioMedicine*, Yin et al. investigate strategies of antigen delivery for optimal cross-presentation (Yin et al., 2016).

Many effective vaccines have been developed against a variety of infections and tumour vaccination is currently receiving renewed interest (Katsnelson, 2016). There have been extensive efforts in improving vaccination strategies. Basic formulations consisting of antigen (peptide or protein) with or without adjuvant elicit some T cell activation but these are usually insufficient to destroy established tumours (Mocellin et al., 2004). Similarly, infusing patient-derived DCs loaded with tumour antigens failed to broadly protect against malignomata, although T cell responses were often observed (Anguille et al., 2014). Much research has been focussed on improving T cell responses by targeting antigens *in vivo* to specific receptors on DCs, mostly by coupling the antigen to an antibody against a DC-specific cell surface molecule. The rationale for this approach is two-fold: first, DCs are targeted and thus antigen is directed to those cells best able to activate T cells; and second, antigen–antibody complexes are delivered to specific receptors on the DC surface resulting in antigen trafficking to MHC-I and/or MHC-II pathways for cross-presentation to CD8 T cells and presentation to CD4 T cells, respectively.

Initial DC targeting studies used the model antigens HEL and Ova conjugated to anti-DEC-205 antibodies resulting in CD4 and CD8 T cell responses in murine models (Hawiger et al., 2001). Since then, a myriad of studies have targeted many different receptors and reported varying degrees of CD4 and/or CD8 T cell activation (Kastenmüller et al., 2014). This has been explained either by different DC subsets expressing the receptor targeted or by different intracellular routing of antigen initiated by the receptor targeted (Kurts et al., 2010). However, very few studies have performed comparative studies on the efficiency of different targeting antibodies with respect to activating CD4 and CD8 T cell responses (Kastenmüller et al., 2014).

In the present study by Yin et al., the authors quantitatively compare the vaccination responses elicited by a panel of antigen-coupled monoclonal antibodies against various human DC surface receptors using polyI:C as adjuvant. For all antigen formulations tested, targeting CD40 was most effective at inducing *in vitro* expansion of antigen-specific CD8 T cells and acquisition of effector function. Targeting antigen to CD40 resulted in a 3–4–fold increase in expansion of antigen-specific CD8 T cells compared to OX-1 targeting, the second best, and was about 1000× better than an equimolar amount of uncoupled antigen. Similar results on CD40 targeting have been reported before (Rosalia et al., 2015). However, the present study identified a hierarchy of CD8 T cell priming efficiency depending on the surface receptor targeted.

An open question for future studies is why targeting CD40 was superior. Several explanations discussed by the authors are conceivable. As previously described (Chatterjee et al., 2012), anti-CD40 Ig remains mostly at the cell surface and traffics to early endosomes with little delivery to late endolysosomes. The slow trafficking of anti-CD40 Ig compared to LOX-1 and Dectin-1 may explain why targeting CD40 resulted in prolonged antigen presentation to CD8 T cells. In addition, antigen accumulation in early endosomes may be crucial for determining its superiority on cross-presentation (Kurts et al., 2010). A second potential explanation may be the expression levels of CD40 on DCs, which the authors propose to be somewhat higher than those of other receptors used, *e.g.* LOX-1 and DEC-205. However, DCIR and CD40 were apparently expressed at similar levels and thus, at least for this pair, expression levels cannot explain why CD40 targeting is superior. A third explanation is that anti-CD40 Ig may activate DCs for better T cell priming. However, the authors argue that targeting antigen with anti-CD40 Ig in a previous report did not result in DC activation (Chatterjee et al., 2012). However, Yin et al. used a different hybridoma

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clone in the present study (12E12) and thus results are difficult to extrapolate. The authors argued that 12E12 did not activate DCs (data not shown), but this possibility remains to be formally excluded. Future studies are warranted to clarify the mechanisms underlying the superiority of CD40 targeting.

What about CD4 T cell responses? Targeting CD40 was not as efficient as other receptors, possibly because CD40 does not recycle into the late endosomal compartments, where MHC-II/peptide complexes are mostly generated (Rocha and Neefjes, 2008). The finding that some receptors are better suited for priming CD4 T cells and others for CD8 T cells may not be surprising given the available literature (Kastenmüller et al., 2014). However, the strength of the Yin et al. study is the comprehensive comparative targeting of an ample set of receptors.

In conclusion, Yin et al. suggest a hierarchy of DC receptor suitability for promoting CD4 and/or CD8 T cell responses. As their study is based primarily on human DCs expanded *in vitro* from blood-derived monocytes, future work is warranted to extend these results to primary human dendritic cells that activate T cells in response to vaccines. This is challenging, but necessary as the CD40 expression profiles differ between the murine and human system. The use of mice with a humanised immune system may help to unveil such complexities, facilitating rational design of optimal vaccines.

Conflicts of Interest

The authors have no conflict of interest.

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