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Improving vaccines by targeting antigens to dendritic cells

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Abbreviations: cDC, conventional DC; DC, dendritic cell/cells; pDC, plasmacytoid DC

Abstract

A new approach to enhancing the effectiveness of vaccines is to deliver antigens selectively to dendritic cells (DC) *in situ*, via monoclonal antibodies specific for particular DC surface molecules. This can markedly enhance CTL responses and, via helper T cells, also enhance antibody responses. DC activation agents or adjuvants must also be administered for effective CTL responses, but in some cases good antibody responses can be obtained without adjuvants. Here we review the role of different DC subsets and different DC target molecules in obtaining enhanced immune responses.

Keywords: adjuvants, immunologic; antibody formation; CLEC9a protein, human; DEC-205 receptor; dendritic cells; vaccines

Introduction

Dendritic cells (DC) are the crucial sentinel cells of the adaptive immune system (Steinman, 1991). They continuously sample the antigenic environment and are sensors of microbial invasion or tissue damage. They not only process and present antigens to activate naïve T lymphocytes, but they also regulate the nature of the T cell response obtained, determining whether it leads to tolerance or to a Th1 or Th2 effector T cell responses. These central roles make manipulation of the DC system an attractive strategy for modulating immune responses.

It is now clear that one of the roles of adjuvants which enhance response to antigens is to activate the DC system; DC themselves have been termed "Nature's Adjuvants". DC have been used in a very direct way in the clinic for the immunotherapy of cancer, so far with only limited success. In this approach DC are produced in culture from the patient's progenitor cells, the cultured DC are loaded with tumor antigens and then injected back into the patient, in the hope of inducing a more effective anti-tumour response (Tacken *et al*., 2007). Even if further refinements increase the success of this approach, it remains complex and expensive. The alternative considered in this review is to target antigens directly to the DC *in situ*, with or without associated DC activation agents. The current approach uses monoclonal antibodies (mAb) specific for molecules on the DC surface to carry linked antigens directly to the DC (Tacken *et al*., 2007). In principle this should reduce the quantity of antigen needed and improve the effectiveness of the vaccine injected. We are particularly interested in enhancing antibody responses by this approach, so that is the main focus of this review.

DC subtypes

Before considering targeting antigens to DC *in situ*, it is important to know about the types of DC which will receive the antigen cargo. The DC normally used for current immune therapy are derived from monocytes stimulated with GM-CSF and IL-4, which models "inflammatory DC", not normally found in steady-state but produced *in vivo* as a response to inflammation (Shortman and Naik, 2007). As an emergency response DC, they may well be appropriate for such DC transfer therapy, but they are not the type of DC which would immediately encounter a targeted vaccine antigen injected into a healthy individual. Several distinct types of DC, differing in origin and specialised functions, are present in steady-state (Shortman and Naik, 2007). One major division is into plasmacytoid DC (pDC, sometimes called lymphoid DC) and conventional DC (cDC, sometimes called myeloid DC). The pDC serve as the major producers of type 1 interferons in response to viral infections, and only assume a dendritic form and antigen-presenting functions after activation; cDC already have dendritic form and antigen uptake and presentation functions. The cDC are of two general types. Migratory cDC, such as the Langerhans cells of the epidermis, begin as antigen col-

lecting cells in peripheral tissues, then migrate through the lymphatics into lymph nodes where they present the antigens to T cells. Lymphoid tissue resident cDC, which arrive in lymphoid tissues as blood-borne precursor cells, carry out both their antigen collecting and antigen presentation functions within the lymphoid organs. Finally, there are functionally distinct subsets of both migratory and tissue resident DCs (Shortman and Naik, 2007; Villadangos and Schnorrer, 2007). In the mouse, the latter can be segregated into $CD8^+$ and $CD8^-$ subtypes. The $CD8^+$cDC are the major producers of IL-12p70 on activation (Reis e Sousa *et al*., 1997; Hochrein *et al*., 2001), so can initiate inflammatory Th1 type responses. They also have a special ability to take up dead cells and other material and cross-present these exogenous antigens on MHC class I (den Haan *et al*., 2000; Pooley *et al*., 2001; Schnorrer *et al*., 2006; Villadangos and Schnorrer, 2007). Thus, although they can activate CD4 T cells, they are especially adept at activating CD8 T cells to produce cytotoxic T cells. The $CD8^-$cDC are more adept at activating CD4 T cells (Dudziak *et al*., 2007; Villadangos and Schnorrer, 2007). These divisions, now well developed for the mouse DC system, are not yet fully established for the human DC system. In particular, $CD8\alpha$ is not expressed on human DC so other markers may serve to distinguish important subsets. There is evidence that the BDCA-3^+ DC subtype of human blood may represent a DC lineage equivalent to the mouse $CD8^+$cDC, since they have several other surface markers in common, including Necl2 (Galibert *et al*., 2005) and Clec9A (Caminschi *et al*., 2008; Huysamen *et al*., 2008; Sancho *et al*., 2008).

Logistics of targeting antigens to DC

Our increasing knowledge of the DC system should provide some guidance to the most effective targeting approach. Logically the DC surface molecule to be targeted should be as DC-specific as possible, to reduce the dose of antigen required. Binding to other cells could "mop up" the injected vaccine, as well as cause unwanted side effects. Unfortunately, there are few if any DC-specific surface molecules; even the mouse "DC-marker" molecule CD11c is expressed by other cells, including macrophages, NK cells and activated CD8 T cells. However, DC are strategically placed within the tissues so as to have selective access to introduced antigens, which compensates somewhat for reduced target specificity. Ideally the surface molecule targeted should be an endocytic receptor, and there is evidence that different receptors can shuttle antigens into different processing pathways (Burgdorf *et al*., 2007). The DC subtype presenting the antigen could determine the type of immune response obtained, so targeting specific DC subtypes should be an advantage; however we do not as yet understand all the rules governing DC subtype responses, especially in the human DC system. If the objective is to improve antibody responses, DC targeting should aim to maximise helper CD4 T cell responses which usually are the limiting factor; however it is unclear whether the eventual antibody producing B-cells should also be targeted, whether a little antigen will suffice for the B cells, or whether the targeted DC can present antigen to the B cells as well as the helper T cells (Wykes *et al*., 1998). Finally, since the eventual aim is to apply this targeting approach to humans, there is an advantage when working with mouse models to choose DC surface molecules common to the human and mouse DC systems.

Clearly there are many complex factors involved in DC targeting and currently it is difficult to predict how they will all play out in practice. At present there is a need to collect experimental data and learn some of the overriding rules, before applying this approach to clinical practice.

The balance between immunity and tolerance

Activation of the immune system can under some conditions lead to immune tolerance rather than active immunity. Finkelman (Finkelman *et al*., 1996) first noted that targeting antigen to the DC surface molecule now called DCIR2, using the mAb 33D1, resulted in tolerance if no DC activating agents or adjuvants were also administered. This effect was then well documented by studies from the laboratories of Steinman and Nussenzweig, who targeted the multilectin receptor DEC-205 or CD205 (Hawiger *et al*., 2001; Bonifaz *et al*., 2002, 2004; Boscardin *et al*., 2006), that it is expressed by the $CD8^+$ cDC of mouse spleen and also by migratory DC in lymph nodes. Administration of antigens linked to mAb against DEC-205 to steady-state DC produced MHC class I and MHC class II presentation and transient antigen- specific T cell proliferation. However, antigen-specific CD4 and CD8 T cells were subsequently deleted and regulatory CD4 T cells (Tregs) were generated (Hawiger *et al*., 2001; Bonifaz *et al*., 2002; Kretschmer *et al*., 2005; Yamazaki *et al*., 2008). The result was a failure to produce immune effector cells and the mice were unresponsive to further antigen cha-

llenge, even with adjuvants. This approach has been shown to prevent the onset and even the progression of autoimmune diseases, by targeting autoantigens to DEC-205 in experimental mouse models of type 1 diabetes (Bruder *et al*., 2005; Mukhopadhaya *et al*., 2008).

If the objective in targeting antigens to DC is to produce a vaccine giving both enhanced immune responses and effective immunological memory, then the factors which induce tolerance must be avoided or negated. The extensive studies on DEC-205 targeting indicated that co-administration of factors which activate DC (such as anti-CD40 or toll-like receptor ligands) permits effective cellular and immune responses to the antigens coupled to anti-DEC-205 mAb (Bonifaz *et al*., 2004; Boscardin *et al*., 2006; Soares *et al*., 2007; Trumpfheller *et al*., 2008). These studies have supported the generalisation that targeting antigens to steady-state, non-activated DC leads to tolerance whereas targeting to DC simultaneously activated with adjuvants produces immunity. As discussed below, this concept fits most of the findings on targeting to produce CTL responses, but it is not always in accord with the studies on antibody responses.

Enhancing CTL responses by targeting antigens to DC

Effective generation of cellular immunity, particularly of CTL, is required for resistance to some viral infections and is a major requirement in tumor immunotherapy. CTL responses have been generated by targeting antigens to a range of DC surface molecules, including DEC-205 (Hawiger *et al*., 2001; Bonifaz *et al*., 2002, 2004), DCIR2 (Dudziak *et al*., 2007; Soares *et al*., 2007), mannose receptor (He *et al*., 2007), Dectin-1 (Carter *et al*., 2006), Clec9A (Sancho *et al*., 2008), CD11c and MHC class II (Castro *et al*., 2008). In all these cases some form of adjuvant or DC activating agent was employed to obtain effective cellular immunity, suggesting this is a general requirement for CTL generation. One apparent exception to this was targeting antigens to the scavenger receptor CD36, where without adjuvants a CD8 T cell response was generated and this was associated with a prevention of tumour growth (Tagliani *et al*., 2008). It needs to be verified that inadvertent activation of DC by microbial products had not occurred, before targeting CD36 can be considered as an exceptional case.

In theory targeting antigens to the specialised "cross-presenting" $CD8^+$ cDC subtype in mice, or its proposed equivalent in the human DC system, should produce optimal CD8 T cell responses and CTL generation. Targeting DEC-205 or Clec9A should accomplish this directly, and they have proved effective for this purpose (Hawiger *et al*., 2001; Bonifaz *et al*., 2002, 2004; Sancho *et al*., 2008). However, $CD8^+$ cDC have a capacity to collect and process antigen from dead cells (Iyoda *et al*., 2002; Schulz and Reis e Sousa, 2002; Schnorrer *et al*., 2006), so even antigens targeted to other DC may eventually be reprocessed by $CD8^+$ cDC. An additional advantage of targeting $CD8^+$ cDC is that these are the major producers of IL-12p70 (Reis e Sousa *et al*., 1997; Hochrein *et al*., 2001), a well established factor for CTL development. However, IL-12p70 is only produced when $CD8^+$ cDC are activated, one reason why adjuvants or DC activation agents are required for CTL generation.

Enhancing antibody responses by targeting antigens to DC

We are particularly interested in improving antibody responses, since there are many situations where possible vaccine antigens give only limited protection against infections because of low antibody production. Our assumption is that helper T cells were the limiting factor, so targeting antigens to DC should enhance the response of CD4 T cells and indirectly improve antibody production. Targeting antigens to many different DC surface molecules has enhanced CD4 T cell responses (Hawiger *et al*., 2001; Bonifaz *et al*., 2002; Bonifaz *et al*., 2004; Carter *et al*., 2006; Dudziak *et al*., 2007; He *et al*., 2007; Soares *et al*., 2007; Caminschi *et al*., 2008; Castro *et al*., 2008). We have found enhanced antibody responses by targeting antigens to the DC surface molecules FIRE, CIRE, Clec9A and Clec12A, and shown this response is dependent on T cells and on MHC class II antigen presentation by DC ((Corbett *et al*., 2005; Caminschi *et al*., 2008); Caminschi, unpublished). Targeting $CD8^-$cDC would seem the most effective strategy for obtaining optimal CD4 T cell responses, and enhanced antibody responses have been obtained by this approach (Corbett *et al*., 2005). However, $CD8^+$cDC are capable of effective CD4 T cell activation despite deflection of much antigen into the MHC class I presentation pathway (Pooley *et al*., 2001; Schnorrer *et al*., 2006), and excellent antibody responses have been obtained by targeting antigen to DEC-205 (Boscardin *et al*., 2006; Soares *et al*., 2007) or to Clec9A (Caminschi *et al*., 2008) which are selectively expressed by $CD8^+$ cDC.

An important issue is whether DC activating agents or adjuvants are required to obtain enhanced antibody responses, as they are for CTL responses. Avoiding the side effects of adjuvants would be a step forward in vaccine design. In the standard setting studies targeting antigens to DEC-205 (Boscardin *et al*., 2006), simultaneous DC activation was required to obtain antibody production, just as for CTL production; our own earlier studies targeting DEC-205 gave the same result (Corbett *et al*., 2005). In line with this finding, adjuvants were required for good antibody production when targeting antigens to Dectin-1 (Carter *et al*., 2006) and DCIR2 (Finkelman *et al*., 1996). However, in a series of other studies targeting antigens to other molecules on DC, strong antibody responses were obtained without using adjuvants; the molecules targeted included MHC class II (Carayanniotis and Barber, 1987); MHC class I, FcRγII (Snider *et al*., 1990), CD45, CD45RA, DCIR-2, CD4 (Skea and Barber, 1993), the mannose receptor (He *et al*., 2007) and from our laboratory CIRE (mDC SIGN), FIRE (Corbett *et al*., 2005) and Clec9A (Caminschi *et al*., 2008). In the latter case the same result was obtained using MyD88$^{-/-}$TRIF$^{-/-}$ mice which are unable to respond to toll-like receptor ligands, so DC activation by contaminating microbial products was not the reason. What are the rules which determine whether an adjuvant is required for antibody production?

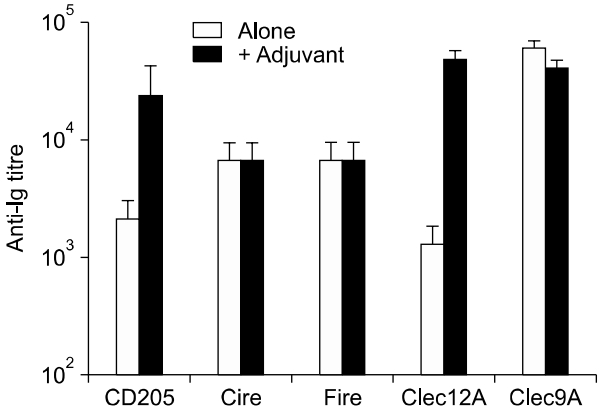

Figure 1. Are adjuvants needed to obtain antibody responses on targeting antigens to DC? The results presented summarise our experience in targeting antigen to DC by intraveneous injection into mice of rat mAb against mouse DC surface molecules. The readout is the mouse antibody response to rat immunoglobulin. In all cases a non-targeting isotype-matched rat Ig gave negligible responses in the absence of adjuvants. Similar results have been obtained for the anti-ovalbumin response when ovalbumin was linked to the targeting mAb. The adjuvants or DC activating agents used have included lipopolysaccharide, poly I:C, CpG and alum; all gave similar effects although the efficiency varied. Although these experiments were not conducted side by side, the results have been consistent once a common sensitive ELISA assay for antibody production was employed. Note that DEC-205 and Clec9A are selectively expressed by CD8$^+$ DC, Fire and Cire are selectively expressed by CD8$^-$ DC, while Clec12A is on all DC but highest on CD8$^+$ DC. Thus the results depend more on the DC surface molecule targeted than the DC subtype.

Our experience on this issue is summarised in Figure 1. With a more sensitive antibody assay we now always see some antibody production on targeting antigens to all DC molecules tested. However, adjuvants markedly enhance antibody production on targeting DEC-205 or targeting Clec12A. In contrast, good antibody production is obtained without adjuvants on targeting antigens to CIRE, FIRE and Clec9A. Since CIRE and FIRE are on CD8$^-$DC and Clec9A, like DEC-205, is on CD8$^+$DC, it seems the subtype of DC targeted is not the determining factor. Rather, the nature of the DC surface molecule targeted determines whether the response requires DC activation. It is possible that DC surface molecules like FIRE, CIRE and Clec9A transmit a subtle signal which, without inducing all the features of DC activation, promotes production of the cytokines required to produce the appropriate helper T cells. Alternatively, these molecules may shuttle antigens into a different processing pathway, perhaps allowing effective antigen presentation to both helper T cells and B cells. The basic mechanism needs to be understood before this promising approach to adjuvant free vaccines can be applied to human populations.

Clec9A as a vaccine

The promise of DC targeting for enhancing the effectiveness of vaccines is well exemplified in recent studies targeting Clec9A. This DC surface molecule, recently described by three laboratories (Caminschi *et al*., 2008; Huysamen *et al*., 2008; Sancho *et al*., 2008), is a C-type lectin-like molecule with a higher specificity for DCs than most DC markers. It is expressed on the CD8$^+$ cDC subtype in mice and the equivalent Clec9A molecule is expressed on the BDCA-3 DC subtype in man, the proposed equivalent of the mouse CD8$^+$ cDC lineage. Several mAb against both the mouse and human Clec9A are available for target studies. Sancho *et al*. (2008) have shown that targeting tumor antigens to Clec9A, along with DC activation agents, promotes T cell responses, CTL production and effective rejection of tumours. Our laboratory has shown that, even without adjuvants, targeting Clec9A promotes the best antibody responses we have obtained (Figure 1) and requires only tiny amounts of the targeting mAb vaccine (Caminschi

et al., 2008). Some clues to why Clec9A is so effective as a target have come from the Reis e Sousa group (personal communication) and from our laboratory (Lahoud *et al*., unpublished). Clec9A serves as a recognition molecule for dead cells. The $CD8^+$cDC, which express Clec9A are especially adept at taking up dead cells and reprocessing dead cell antigens (Iyoda *et al*., 2002; Schulz and Reis e Sousa, 2002; Schnorrer *et al*., 2006). Thus targeting antigens to Clec9A via anti-Clec9A mAb appears to shunt antigens directly into a normal biological process for efficient processing of exogenous antigens. The existence of the equivalent CLEC9A molecule on the surface of a specific subtype of human DC (Caminschi *et al*., 2008; Huysamen *et al*., 2008; Sancho *et al*., 2008) suggests that, once the mouse model studies are complete, translation to human trials could be rapid.

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