Controlling immune responses by targeting antigens to dendritic cell subsets and B cells

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Received 1 October 2013, accepted 18 October 2013

Abstract

Delivering antigens in vivo by coupling them to mAbs specific for unique receptors on antigen-presenting cells (APCs) is a promising approach for modulating immune responses. Antigen delivery to receptors found on myeloid dendritic cell (DC) subsets, plasmacytoid DCs and B cells has shown them all to be viable targets to stimulate either the cellular or humoral arms of the immune system. It is now evident that antigen-targeting approaches can also be used to invoke antigen-specific inhibition of immune responses. The outcome of activation versus inhibition is determined by a combination of factors that include the choice of APC, the receptor that is targeted, whether to include an adjuvant and, if so, which adjuvant to employ. In addition to their use as a means to modulate immune responses, antigen-targeting systems are also a useful method to investigate the function of DC subsets and the early mechanistic events that underlie the initiation of both cellular and humoral immune responses. In this review, we focus on the literature surrounding the control of B-cell responses when antigen is delivered to various APC subsets.

Keywords: antibody, antigen targeting, B cell, dendritic cell

Introduction

Adaptive immune responses are initiated by antigen-presenting cells (APCs) that acquire antigens from their environment and present them to B and T lymphocytes. APCs are a heterogeneous group of cells that include macrophages, B cells, myeloid dendritic cell (DC) subsets and plasmacytoid DCs (pDCs). Because of the key role of APCs in initiating immune responses, multiple strategies have been devised to deliver antigens directly to one or more APC subsets; strategies include conjugation of antigens to ligands or mAbs specific for select immunoreceptors, using antigen-containing nanoparticles, using pseudotyped lentiviruses and using recombinant adenoviruses (1). Because of their availability, high selectivity, high affinity and avidity, mAbs have been the preferred choice for most antigen-targeting studies.

Delivering antigens to specific APC subsets is an attractive approach for manipulating antigen-specific immune responses because it can increase the efficacy of the immunization and, importantly, allow control over which cell type(s) present antigens to the immune system. As our knowledge of the function of APC subsets has grown, this latter point becomes increasingly important for rationally designing immunization strategies aimed toward raising specific types of immune responses (e.g. CD4⁺, CD8⁺ or B-cell responses), or conversely, promoting a state of immunological unresponsiveness, (e.g. tolerance).

The concept of using mAbs to deliver antigens to receptors on immune cell subsets for the purpose of stimulating immune responses was established over 25 years ago with the delivery of antigen to B cells using anti-immunoglobulin monoclonal antibodies (2). Since then, however, DCs and their subsets have become a major focus of antigen targeting because of their heightened ability to activate naive T-cell subsets. As a result, most studies have focused on the generation of CD4⁺ and CD8⁺ T-cell immunity. However, DCs can also act as APCs for B cells and induce them to produce antibodies (3–7). With this knowledge, a select number of receptors have been targeted on DCs that lead to robust antibody responses, even in the absence of adjuvants. Unlike antigen processing and presentation to T cells, the pathways that dictate antigen preservation and presentation to B cells and their activation are not as well defined.

Delivering antigen directly to distinct cell types and surface receptors has opened up the ability to manipulate B-cell responses and allowed the opportunity to investigate pathways by which B cell and antibody responses are initiated or inhibited. In this review, we discuss the literature surrounding the initiation and control of B-cell responses when antigen is delivered to APC subsets and their receptors.
Targeting antigens to B cells

It is well established that B cells can efficiently take up, internalize and process antigens through their high-affinity surface BCRs; thus, some of the first immunotargeting studies used mAbs specific for IgM, IgD or IgG. Kawamura and Berzofsky (2) showed that ferritin coupled to anti-IgG antibodies was more immunogenic in vivo than free ferritin. Later studies confirmed this finding for anti-IgM and anti-IgD mAbs as well (8, 9). However, since circulating IgM or IgG is also presumably bound by antigen attached to anti-IgM or anti-IgG, it is not clear how or if antigen coupled to anti-IgM or anti-IgG can efficiently enter the immune system. Since little or no free IgD is in circulation, surface IgD (slgD) is a more attractive choice to target (9); however, several groups have reported that targeting to slgD is not as effective as targeting to other surface molecules such as MHC class II (8).

Monoclonal antibody-based antigen targeting in vivo without adjuvant was pioneered by Barber and co-workers using mAbs specific for MHC class II (10), and confirmed by others (8). Antigen bound to anti-MHC class II is taken up efficiently and processed (8, 11), but why targeting to MHC class II is so effective is not clear. It could be due to a combination of factors including: (i) prolonged retention of antigen once it is bound and processed via MHC class II (12), (ii) the induction of co-stimulatory molecules like CD80/CD86 after MHC class II ligation (13), (iii) the fact that MHC class II is expressed on all APCs and/or (iv) a signaling pathway similar to the BCR signaling pathway being induced via MHC class II (14).

Following their initial success with antigen targeting, Barber and his colleagues compared immune responses induced after targeting to MHC class II versus other receptors (15). Overall, they concluded, as did subsequent studies, that when antigens are targeted to the more broadly expressed receptors such as MHC class II and CD11c, stronger antibody responses are induced than when antigens are targeted to receptors relatively restricted to B cells (e.g. slgM, slgD, B220, FcγRIIB, CD22 and CD19) (8, 15–17). It is difficult, however, to make firm conclusions based on these early studies since mAbs of different isotypes or from different species were used and/or compared. Thus, some results could be due to effects of differential binding to FcRs, variation in monoclonal antibody affinities or the immunogenic epitopes within the monoclonal antibodies themselves.

More recently, our laboratory has targeted antigens to the TLR family member CD180 [RP105 (radioprotective 105 kDa)]. CD180 is closely related to TLR4 (61% sequence similarity) and like TLR4, which forms a heterodimer with myeloid differentiation 2 (MD-2), CD180 forms a heterodimer with MD-1 that is required to associate with CD180 for the complex to be expressed on the cell surface (18, 19) (Fig. 1). No ligand for CD180 has yet been identified, and the structure of MD-1 differs from that of MD-2, suggesting it does not bind LPS (20). Unlike other TLR family members, CD180 does not have a Toll/IL-1R (TIR) domain but, nevertheless, ligating CD180 leads to receptor internalization and signaling.

We selected CD180 as a target for induction of antigen-specific B-cell responses because: (i) CD180 is relatively restricted to B cells and myeloid cells (21), (ii) cross-linking CD180 triggers a signaling pathway similar to that induced by BCR ligation and drives B cells to enter the cell cycle (22, 23) and (iii) mAbs to CD180 activate B cells, and injecting mice with high doses of rat anti-CD180 induces polyclonal B-cell activation and increases polyclonal IgG levels (24).

Mice injected with anti-CD180 to which hapten, protein or viral envelope antigens had been attached rapidly developed antigen-specific IgG antibody without the addition of an adjuvant (C. Dresch and K. E. Draves, unpublished data) (25). The IgG responses induced by targeting to CD180 were stronger and more rapid than in mice immunized with antigen in alum. Using the nitrophenol (NP) hapten system, we found that after one injection of NP-anti-CD180, high-affinity antibodies were generated within 7 days and that immunologic memory including IgG-producing antibody-forming cells was induced in both wild-type and CD40-deficient mice. Surprisingly, strong IgG antibody responses were even induced by antigen-anti-CD180 immunization in mice deficient in mature B cells [i.e. B-cell-activating factor receptor (BAFF-R) knockout (KO) mice], mice deficient in signaling via type I interferons (i.e. IFN-α/βR KO mice), OX40 ligand KO mice and IL-4 KO mice (25). It is remarkable that CD180 induces antibody responses in these animals because these proteins are required for either normal B-cell development and/or the generation of robust, T-cell-dependent (TD) IgG antibody responses in the presence of conventional adjuvants. The fact that CD180 circumvents these requirements suggests that targeting antigens to CD180 may be useful for inducing protective immunity in immunodeficient populations.

For a strong and rapid antigen-specific IgG response to be induced, antigens must be attached to anti-CD180 and...
CD180 has to be expressed on B cells, but does not have to be expressed on non-B cells (e.g. DCs). The combination of signals via CD180 and antigen-engaged BCRs appears to be responsible for the effectiveness of this immunization method (Fig. 1). CD180-based antigen targeting also leads to antigen processing by DCs, which then in turn can activate both CD4+ and CD8+ T cells (C. Dresch, unpublished data) (25). Furthermore, mice given one injection of West Nile virus (WNV) envelope protein coupled to anti-CD180 survived a normally lethal intracranial infection by WNV (C. Dresch, unpublished data).

In contrast to raising antibody responses, Macauley et al. (26) recently demonstrated that B-cell responses can be inhibited by coupling antigens to a ligand for CD22, an ITIM-containing receptor on B cells that regulates BCR signaling. Mice inoculated with complexes of protein antigen plus CD22 ligand (CD22L) as a liposomal nanoparticle formulation developed antigen-specific B-cell tolerance to a number of different antigens. Liposomes containing only CD22L did not globally suppress B-cell responses, and antibody responses were actually induced when CD22L was not included in the formulation, demonstrating that simultaneous signaling via CD22 and the BCR was required for tolerance induction. Macauley et al. further showed that nanoparticle formulations containing clotting factor VIII (FVIII) plus CD22L could reduce the development of inhibitory anti-FVIII antibodies in hemophilic mice treated with FVIII and prevent bleeding. These studies, together with our findings from targeting CD180, demonstrate the impact that co-stimulatory signals have in determining how B cells respond when they recognize antigen via the BCR.

The ability of secondary signals to modulate antibody responses following antigen targeting is also evident when targeting antigens to CD11c, which is expressed at high levels primarily by myeloid DCs. Glennie et al. (17, 27) used intravenous immunization to target ovalbumin (OVA) to receptors expressed on DCs by conjugating the antigen to Fas ligand, which eliminates effects that are due to FcR binding or extensive receptor cross-linking. In particular, anti-CD11c coupled to antigen was highly effective at inducing both T-cell and antibody responses in vivo. This appears to not simply be due to the tissue distribution of CD11c since targeting of pan-APC surface molecules like MHC class II was less effective. Furthermore, anti-CD11c alone could act as an adjuvant to enhance antibody responses when antigen was concurrently targeted to separate receptors on DCs, for example, DC immunoreceptor 2 (DCIR2) and CD11b, but not when antigen was targeted to CD21 or CD19 on B cells (17).

This suggests that anti-CD11c is highly effective at targeting both because of the tissue distribution of CD11c and because of the signaling properties of anti-CD11c, which, for instance, can promote B-cell activation and proliferation (28). Antigen targeted via CD11c was found primarily in the splenic red pulp and marginal zone (MZ) where CD8α+ DCs reside. Thus, it is quite possible that the strong adjuvant effect of antigen–anti-CD11c works because it induces a signaling pathway in CD8α+ DCs, a DC subset that is poised and highly effective for B-cell activation and antibody responses (see below). Consistent with this model is the fact that anti-CD11c alone enhances antibody responses when antigen is targeted to DCIR2, which is only expressed on CD8α+ DCs (17). Interestingly, targeting antigen to CD11b appears to be less effective than targeting to CD11c, even though both these receptors are co-expressed on many myeloid cells and up-regulated by inflammation (15, 17). Again, this suggests CD11c may trigger a distinct signaling/processing pathway in certain DCs.

Collectively, the data suggest that targeting to CD11c and CD180 is effective without adjuvants because the antigens are processed via CD8α+ DCs for presentation to B cells (discussed below) and/or because the antigens are processed directly by B cells under conditions that activate B cells to become effective APCs for T cells.

Antigen targeting to myeloid DCs

**DC-induced antibody responses: how do they work?**

A number of studies has shown that DCs can influence a range of B-cell processes including proliferation, differentiation and immunoglobulin class-switch recombination (5–7, 29–32). Indeed, antibody production can be induced after targeting antigens to any of a growing list of receptors on DCs including CD11c, C-type lectin lectin 12a (Clec12a), dendritic and epithelial cell receptor of 205 kDa (DEC205), DC-associated C-type lectin 1 (Dectin 1), C-type lectin immunoreceptor (CIRE), f4/80-like receptor (FIRE), DCIR2, Clec9a, MHC class I and II, and murine DC immunomodulating receptor 1 (mDCAR1) (4, 10, 16, 17, 33–38).

Because many of these receptors are found on multiple APC subsets, it is difficult to determine with certainty the cell type and mechanism that is responsible for induction of antibody responses. However, several receptors have been targeted that are relatively restricted to a single DC subset in the mouse, and from these studies some commonalities can be gleaned concerning DC-induced humoral immune responses. They include CIRE, which is the mouse homolog of DC-SIGN (DC-specific intercellular adhesion molecule 3-grabbing non-integrin), FIRE, Dectin 1 and DCIR2 on DCs (4, 16, 34, 36, 39), and DEC205, Clec9a and DCAR1 on CD8α+ DCs (35, 37, 40). Among these, the receptors that yield robust TD antibody responses in the absence of adjuvants are receptors whose expression is restricted to the CD8α+ DC subset (DCIR2, CIRE and FIRE), with the exception of Clec9a that is expressed predominately on CD8α+ DCs. The questions that arise from this observation are: (i) how do DC-mediated antibody responses occur in the absence of adjuvant? and (ii) is the same mechanism used by both CD8α- and CD8α+ DCs?

**B-cell activation and migration**

Our laboratory has investigated antigen-specific B-cell responses following antigen delivery to DCIR2 on CD8α- DCs and shed some light onto the events that lead to antibody responses following antigen targeting to CD8α+ DCs (4). By tracking the fate of antigen-specific B cells in situ, we showed that targeting antigen to DCIR2 resulted in rapid DC-mediated activation of B cells via direct DC–B-cell interaction. Antigen-specific B cells up-regulated numerous activation and co-stimulatory molecules, displayed changes in chemokine receptor expression and accumulated in MZ-associated bridging channels where they acquired antigen from DCIR2+ CD8α+ DCs as early as 24 h following antigen uptake via DCIR2. Subsequently, antigen-specific B cells were found...
along T–B borders, and then later they migrated to the red pulp and underwent plasma cell differentiation.

Collectively, the results suggest a stepwise model for the events that occur following antigen targeting to CD8α− DCs (Fig. 2): (i) antigen-targeted DCIR2+ DCs capture, internalize and recycle antigen to the cell surface, (ii) presentation by DCs of antigen to antigen-specific B cells (likely emerging from circulation) in MZ-associated bridging channels, (iii) activated B cells in turn process and present antigen on MHC class I to antigen-specific CD4+ T cells at T–B borders and (iv) activated CD4+ T cells proliferate and cross-talk (co-stimulation) between the T and B cells results in B-cell differentiation to plasma cells.

The model for the events that drive antibody responses following antigen delivery to CD8α− DCs may not be applicable to antigen targeting to Clec9a on CD8α− DCs, which facilitates robust TD antibody and germinal center (GC) responses in the absence of adjuvant (35, 41). CD8α− DCs are primarily found in the T-cell zones, and to a lesser extent within MZs (39, 42). This is distinct from that of DCIR2+ CD8α− DCs, which are predominantly located in the bridging channels associated with MZs (39). It is unclear whether CD8α− DCs directly present antigen to B cells following antigen delivery to Clec9a. Targeting antigen to DEC205 on CD8α− DCs fails to induce direct B-cell activation and antibody responses (4), although including CpG does allow for induction of antibody (CpG binds TLR9 on B cells, myeloid DCs and pDCs) (36, 43). Thus, it appears that antibody responses driven by CD8α− DCs may occur via a mechanism distinct from that of CD8α+ DCs.

Lahoud et al. propose that B cells may acquire their antigen from persisting, free antigen-monoclonal antibody complexes in the circulation. Unlike anti-DEC205-antigen complexes, anti-Clec9a-antigen complexes persist in the bloodstream for 4 days following injection, allowing ample time for B cells to acquire antigen via their BCR (44). Although binding small quantities of antigen conjugated to a non-targeted isotype control monoclonal antibody via the BCR is insufficient for overt B-cell activation (4), it nevertheless may provide B cells with a source of antigen to present to previously activated CD4+ T cells. Alternatively, if the location of CD8α− DCs (MZs and bridging channels) is in fact a critical factor in their ability to engage B cells and induce antibody responses, then a similar process could be operative for the subset of CD8α+ DCs also found in MZs (42).

A remarkable feature of Clec9a antigen targeting is the robust generation of CD4+ follicular helper T cells (Tfh cells) (44), which are required for both extralymphatic plasma cell and GC responses to TD antigen (45). After targeting antigen to Clec9a, such a strong CD4+ T-cell response may be sufficient to drive B cells to differentiate to both plasma cells and GC B cells. Although further studies are required to more precisely define the cellular events that unfold during Clec9a-induced antibody responses, the current data suggest that CD8α- and CD8α+ DCs utilize different mechanisms to invoke humoral responses: CD8α− DCs induce strong activation of B cells that drive CD4+ T-cell expansion, whereas CD8α+ DCs induce strong CD4+ T-cell expansion and differentiation that in turn drives B-cell differentiation.

Why are CD8α− DCs seemingly more efficient at inducing antibody responses compared with CD8α+ DCs in the absence of adjuvant? Humoral responses to most protein antigens require the generation of helper CD4+ T cells, particularly B-cell lymphoma 6 (BCL6)− Tfh cells (45). The two major myeloid DC subsets—CD8α+ and CD8α− DCs in mice—appear to have distinct functional differences with regard to priming T-cell subsets (39, 46, 47).

These differences can be attributed, in part, to the selective expression of genes required for antigen processing and presentation. CD8α− DCs are enriched in gene expression for MHC class II-associated genes such as lysosomal hydrolases including cathepsins Z, H and C, whereas CD8α+ DCs have high expression of proteins required for MHC class I antigen presentation including peptide transporters TAP1 and TAP2 (39). Thus, the ability of CD8α− DCs to preferentially process
antigens for MHC class II presentation and CD4+ T-cell activation may be one advantageous quality that allows them to assist in induction of antibody responses.

The robust CD4+ T-cell responses following Clec9a antigen targeting, however, clearly show that the disparities in antigen presentation between DC subsets are not absolute. The decision to present antigens on MHC class I or II depends on multiple factors including the type of DC activation stimulus as well as the receptor that binds and internalizes the antigen (48–50). Antigen delivered to early endosomes via anti-CD40, for instance, facilitates strong cross-presentation (i.e. presentation of exogenous antigen by MHC class I) by the subset of human DCs expressing blood DC antigen 1 (BDCA1), but not if antigen is delivered to DEC205 (49, 51). Conversely, antigen internalized by Clec9a or mDCAR1 on CD8α+ mouse DCs leads to robust CD4+ and CD8+ T-cell responses, which in the case of mDCAR1 was shown to be a result of antigen shuffling to both the cross-presentation pathway and late endolysosomes (35, 37, 44, 52).

In addition to transporting antigen to late endolysosomal compartments for presentation on MHC class II, the receptor must also preserve the antigen’s native, tertiary structure for recognition by B cells. When antigen is taken up via the BCR, signaling via ITAM-binding protein tyrosine kinases (PTKs) is required for it to be processed and presented by MHC class II (53). Similarly, antigen within immune complexes (ICs) taken up by FcγRs on DCs utilizes an ITAM pathway for processing and presentation by MHC class II (3). However, when antigen within ICs is taken up by ITIM-containing FcγRs on DCs, it is preserved, enters recycling endosomes and is presented in an intact form on DC surfaces to B cells in a process known as antigen recycling (3). DCIR2 also contains an ITIM which, as discussed at the beginning of this section, is a highly effective target for bringing about DC–B-cell cell interactions (4). In contrast, antigen targeted to the mannose receptor is efficiently processed for cross-presentation by MHC class I (54).

Thus, the outcome of antigen targeting can differ depending on the signaling elements associated with the targeted receptor. Given their propensity for inducing B-cell responses, it is interesting to speculate that CD8α+ DCs have a greater capacity, or greater number of unique receptors, that direct cargo to the antigen-recycling pathway compared with CD8α+ DCs.

**Targeting antigens to pDCs**

Initially, pDCs were identified as natural interferon-producing cells because of their ability to secrete high quantities of type I interferons following treatment with either TLR7 or TLR9 agonists (55). Phenotypically, mouse pDCs are characterized by expression of CD45RA/B220, intermediate expression of CD11c and high expression of bone marrow stromal antigen 2 (BST-2 or PDCA-1 or CD317), sialic acid-binding immunoglobulin-like lectin H (Siglec-H), Ly6C and Ly49Q; human pDCs lack CD11c and express high levels of BDCA2 (also called CD303 or Clec4C), immunoglobulin-like transcript 7 (ILT7; CD123), DCIR and BDCA4.

In contrast to myeloid DCs, the ability of pDCs to directly mediate T-cell activation and proliferation has been more controversial (56). Several recent studies have shown that pDCs can activate CD4+ T cells to expand and differentiate into Th1, Th2 or Th17 cells particularly when pDCs are stimulated with TLR agonists (57, 58). Similar to myeloid DCs, pDCs can also induce T-cell tolerance by promoting Treg cells and inducing T-cell unresponsiveness (57, 59). Experiments in which pDCs were selectively depleted by treating Siglec–H–DTR (diphtheria toxin receptor) transgenic (Tg) mice with diphtheria toxin convincingly implicated pDCs in CpG-A-driven toxic shock syndrome and in peripheral tolerance generated by antigen-specific Treg cells (60). The factors that dictate the path of CD4+ T-cell differentiation by pDCs are complex and not fully understood, but can be influenced by pDC maturation status, the type of stimuli (e.g. a TLR agonist) and dose of antigen (61).

Although both myeloid DCs and pDCs can present antigen to and activate T cells, they have distinct modes of antigen processing and presentation (62). Several pDC receptors including BST-2, Siglec-H, BDCA2, DCIR and FcγRII have been shown to mediate endocytosis, processing and presentation of antigens (63–67). More recently, several of these receptors have been analyzed for their ability to modulate immune responses.

**Targeting antigens to BST-2**

BST-2 is expressed at high levels on pDCs. Although it has been used as a pDC marker, BST-2 is also expressed at lower levels on myeloid DCs, macrophages and T cells and is increased after cell activation (68). Furthermore, BST-2 is expressed on inflammatory DCs that are functional APCs (69). Nevertheless, antigen coupled to anti-BST-2 may selectively target antigens to mouse pDCs in vivo (65). The BST-2-targeted pDCs process and present antigens efficiently and induce both CD8+ and CD4+ T-cell expansion even without adjuvants (Fig. 3). The addition of either CpG oligonucleotides (a TLR9 agonist) or poly-I:C (a TLR3 agonist) adjuvants together with OVA–anti-BST-2 immunization promoted more robust T-cell expansion than without adjuvants and also generated OVA-specific IgG antibodies and protective immunity against an OVA-containing virus and an OVA-containing tumor (65). It was not reported whether IgG antibodies could be induced without adjuvant.

**Targeting antigens to Siglec-H and BDCA2**

Siglec-H is expressed on the surface of pDCs in mice, and is a relatively reliable marker for mouse pDCs even though it is also expressed within MZ macrophages (70, 71). Targeting antigens via Siglec-H on pDCs, unlike targeting to BST-2, led to retention of peptide–MHC complexes on pDCs for at least 8 days but did not induce IgG antibody responses, even in the presence of adjuvants (66). And, unlike targeting to DEC205, targeting to Siglec-H, although able to prime and induce proliferation in antigen-specific CD4+ T cells and CD8+ T cells (66, 71), did not induce increases in Foxp3+ Treg cells (Fig. 3). Rather, targeting to Siglec-H impaired antigen-specific T-cell responses to soluble antigen and reduced CD4+ T-cell differentiation to Th1, Th2 or Th17 cells.

BDCA2 is a type II C-type lectin whose expression in humans is restricted to pDCs (63). Upon cross-linking with anti-BDCA2 monoclonal antibody, BDCA2 internalizes into
MHC class I-containing endosomes, induces release of intracellular free Ca^{++}, and processes and presents antigens on MHC class II (72). These attributes make BDCA2 a strong candidate for antigen delivery to pDCs *in vivo*.

There is no mouse homolog for human BDCA2; thus, we established human BDCA2 Tg mice that express BDCA2 selectively in mouse pDCs. Delivery of OVA to pDCs using anti-BDCA2 mAbs (OVA–anti-BDCA2) resulted in a significant reduction in the numbers of OVA-specific Foxp3^+ T reg cells (C. P. Chappell, N. V. Giltiay, K. E. Draves *et al.*, submitted for publication). Upon re-challenge with OVA in alum, mice previously treated with only 1 μg OVA–anti-BDCA2, unlike controls, had a dramatic reduction in OVA-specific IgG production. The BDCA2-induced antigen-specific tolerance was reversed by treating tolerized mice with anti-CD25 to reduce T reg cell numbers prior to re-challenge with OVA in alum, demonstrating that T reg cells are likely required for the BDCA2-induced tolerance (Fig. 3).

**Fig. 3.** Modulation of immune responses by targeting pDCs. Inhibition of IFN-α induced by a variety of stimuli can be achieved by cross-linking Siglec-H or BDCA2 on pDCs. DAP12-mediated signaling upon cross-linking Siglec-H leads to IFN-α inhibition, whereas the adaptor chain FcγRIγ is required for inhibition of IFN-α by BDCA2. Antigen uptake by these receptors leads to a diminution of effector CD4^+ T-cell responses and, in the case of BDCA2, maintenance of T reg cells. In contrast, antigen uptake by BST-2/pDCA-1 leads to immune activation accompanied by increased CD4^+ and CD8^+ T-cell responses as well as antibody production if adjuvant is included at the time of immunization. Unlike Siglec-H and BDCA2, cross-linking BST-2 promotes IFN-α production by TLR ligands, which may further enhance both T- and B-cell responses.

Why does targeting to BST-2, Siglec-H and BDCA2 on pDCs lead to three qualitatively distinct immune responses? Importantly, all three receptors appear to regulate IFN-α production by pDCs. BST-2-deficient pDCs make less IFN-α in response to CpG-A or viruses (73), implying that BST-2 is required for pDCs to efficiently respond to TLR agonists and produce IFN-α. In contrast, ligating either Siglec-H or BDCA2 with mAbs, or in the case of BDCA2 its natural ligand, reduces pDC responses to TLR agonists and IFN-α production through a ITAM-dependent pathway (63, 70, 74). Furthermore, Siglec-H-deficient pDCs make more IFN-α after CpG stimulation (60). Thus, the ability of targeting to BST-2 on pDCs to efficiently promote protective immune responses may be due to BST-2’s function in enhancing IFN-α production and/or antigen processing, whereas Siglec-H and BDCA2 may promote tolerance by inhibiting IFN-α production (Fig. 3).

Evidence thus far suggests that the BDCA2- and Siglec-H-signaling pathways have similarities yet are distinct. Siglec-H interacts with the intracellular adaptor DAP12 (DNAX-activating protein of 12kDa), whereas BDCA2 interacts with the FcγRIγ chain (70, 75). Both DAP12 and FcγRIγ are ITAM-containing adaptors that share in common the activation of a PTK pathway that inhibits interferon regulatory factor 7 (IRF7)-dependent production of IFN-α (76). Upon cross-linking, BDCA2 induces rapid Ca^{++} influx and phosphorylation of multiple signaling molecules including Syk, Src-family PTKs and Erk1/2 (63, 75). Furthermore, CD2-associated adaptor protein (CD2AP) can form a complex with SHIP1 and positively regulate the BDCA2-induced ITAM-signaling pathway (76). The signaling pathway(s) downstream of Siglec-H/DAP12 that mediates inhibition of IFN-α have not been defined.

The unique functions of pDCs in protective immunity, inflammatory responses and tolerance have largely been attributed to their location, their unique ability to secrete significant levels of IFN-α and their high turnover of MHC class II. The ability to modulate IFN-α production by pDCs via Siglec-H and BDCA2 may be a key component to tolerance induction when targeting antigens to these receptors. Cross-linking of other receptors expressed on human pDCs such as DCIR, ILT7 and NKp44 can also block the ability of pDCs to produce type I interferons (55, 64), although the ability of ILT7 and NKp44 to mediate antigen endocytosis and presentation has not been reported. Overall, targeting antigens to pDCs may be a promising method for inducing antigen-specific tolerance and regulating autoimmune or antidrug antibody responses.
Conclusions and future directions

Nearly 20 years after the first immunotargeting studies were reported, surprisingly little is known about how targeting works without adjuvants. Future studies of antigen targeting are likely to uncover currently poorly understood pathways that promote B-cell differentiation. Once the modes of action of certain antigen-targeting strategies are better understood, it may become possible to select adjuvants that activate complementary or synergistic pathways to be used in an optimal combination together with an antigen-targeting platform.

It will also be important to better understand the mechanism(s) of adjuvant-induced enhancement of antigen targeting. Many of the adjuvants used in antigen-targeting systems are agonists of TLRs or other pattern recognition receptors that are abundantly expressed on APC subsets. However, expression of a particular agonistic receptor does not guarantee the adjuvant will enhance an antibody response. Coinjecting CpG when targeting antigen to DEC205 allows expression of a particular agonistic receptor to maintain or enhance T

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Antigen delivery to immune cell subsets


Antigen delivery to immune cell subsets


