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New insights into the role of 4-1BB in immune responses: beyond CD8+ T cells

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Since the discovery of 4-1BB 15 years ago, the receptor and its ligand (4-1BBL) have remained enigmatic molecules because of their highly regulated pattern of expression. Classically, 4-1BB is known to function as a costimulator for T cells and as a potent survival factor for CD8+ T cells. Recent studies highlight the participation of 4-1BB and its ligand 4-1BBL in a more complex network of immune cell responses and suggest that intervening in the 4-1BB costimulatory pathway might have some potential as a therapeutic approach to immune disorders.

Dendritic cells (DCs), the prototypical antigen-presenting cells (APCs), capture antigens and migrate to the secondary lymphoid organs where the antigens are recognized by T cells through the interaction between the T cell receptors (TCRs) and antigen-derived peptides in the major histocompatibility complex (MHC) molecules on the surface of the DCs. During this process DCs convey not only antigen specificity but also other 'packets of information on costimulatory and cytokine identity' to T cells [1]. A variety of pairs of costimulatory receptors on T cells and their ligands on DCs are required for full activation of T cells, which ultimately leads to the generation of effector T cells. Generally, costimulatory receptors can be classified into two families: the CD28/ICOS (inducible costimulator) family and the tumor necrosis factor (TNF) receptor family, to which 4-1BB belongs. Recent findings provide new insights into the role of 4-1BB in immune responses *in vivo*, which might have a potential application as immunotherapy in the clinical setting.

4-1BB as a DC activator

4-1BB is expressed on activated CD4+ T cells, CD8+ T cells, natural killer (NK) cells and NK T cells (reviewed in [2]). CD4+CD25+ regulatory T cells appear to express 4-1BB constitutively [3,4]. Recent studies have shown that 4-1BB expression is not restricted to subpopulations of lymphoid cells but is distributed across a variety of cells. For example, myeloid cells, including monocytes, neutrophils and DCs, express 4-1BB constitutively [2,5–8] and eosinophils express 4-1BB in response to a soluble factor released by activated T cells [9]. The ligand of 4-1BB (4-1BBL) is expressed on activated APCs, such as DCs, B cells and macrophages [2], and on several leukemia cell lines [6]. This expression pattern raises the possibility that 4-1BB and 4-1BBL could be involved in multiple steps in various innate and adaptive immune responses.

Of special note is the fact that, like CD40 and RANK (receptor activator of NF-κB), 4-1BB is one of the DC-activating molecules, in that signaling via 4-1BB results in cytokine production [e.g. interleukin-6 (IL-6) and IL-12] [6,7] and upregulation of costimulatory molecules B7-1 and B7-2 [6]. However, unlike CD40 and RANK, which are triggered by their respective ligands on activated T cells, 4-1BB probably receives its stimulus from adjacent APCs (including the DCs themselves), rather than from T cells [7]. Although the specific consequences of DC activation through

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4-1BB signaling remain to be determined, it is possible that activated DCs are, in turn, capable of activating both CD4+ and CD8+ T cells through the 4-1BB–4-1BBL signaling pathway, suggesting that 4-1BB might have a role in both CD4+ T-cell-mediated and CD8+ T-cell-mediated immune responses (Fig. 1).

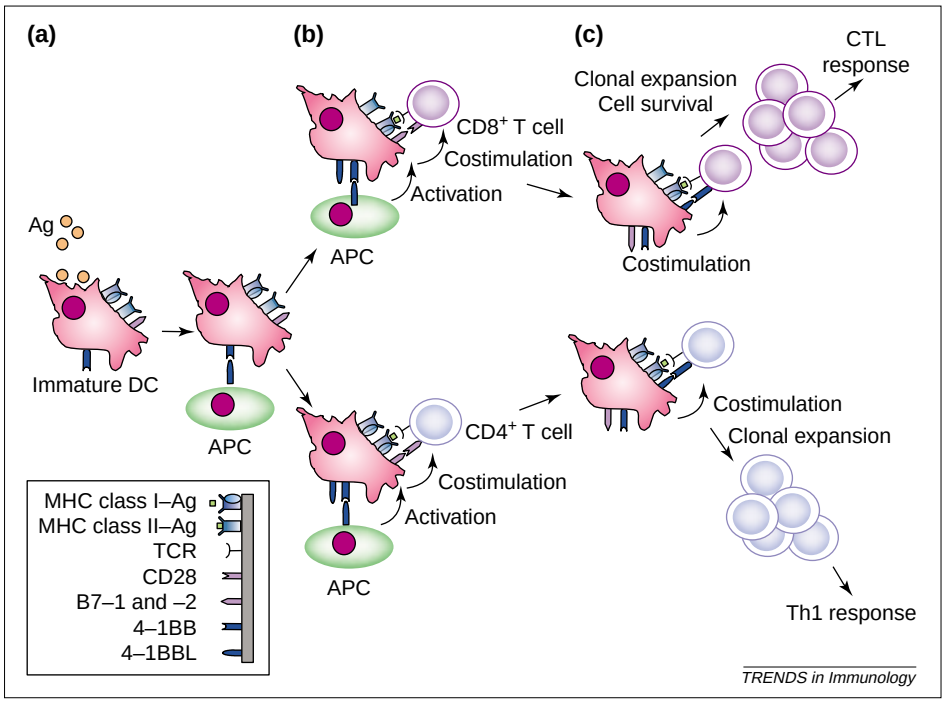

Fig. 1. Schematic illustration of the role of 4-1BB in dendritic cell (DC) and T-cell activation. (a) After capturing antigens (Ags), immature DCs undergo maturation and migrate to secondary lymphoid organs. Mature DCs express 4-1BBL and can be further activated through 4-1BB, which might be triggered by 4-1BBL on the surface of as-yet-unidentified cells (possibly APCs). (b) DCs then secrete cytokines and upregulate B7-1 and B7-2, which, in turn, provide initial costimulatory signals via the CD28 receptor, inducing the expression of 4-1BB on T cells. (c) Once 4-1BB is expressed on T cells, signaling through 4-1BB sustains the initial CD28-signal-mediated activation and further enhances clonal expansion and survival of CD8+ T cells. In the case of CD4+ T cells, signaling through 4-1BB seems to induce clonal expansion but apparently does not induce the prolonged survival seen in CD8+ T cells.

4-1BB regulates the clonal expansion and survival of CD8+ T cells

In a seminal paper, Shuford *et al.* demonstrated that signaling through 4-1BB preferentially induces proliferation of CD8+ T cells [10]. Later, Takahashi *et al.* demonstrated that 4-1BB stimulation significantly increases the survival of superantigen-activated CD8+ T cells *in vivo* [11].

Two recent papers strongly support 4-1BB regulation of both clonal expansion and survival of CD8+ T cells *in vitro* and *in vivo* [12,13]. Maus *et al.* developed artificial APCs that induce proliferation of CD8+ T cells and used them in an *ex vivo* coculture system to demonstrate the remarkable growth and survival of CD8+ T cells achieved by TCR, CD28 and 4-1BB stimulation compared with TCR and CD28 stimulation [12]. These findings and the observation that naïve CD8+ T cells initially activated through TCR/CD28 signals become refractory to repeated TCR/CD28 stimulation suggest the need for costimulatory signaling via 4-1BB to sustain the response (Fig. 1). The promotion and prolongation of CD8+ T cell proliferation and survival by 4-1BB stimulation appears to be mediated, at least in part, through increased production of IL-2 and expression of Bcl-xL, an anti-apoptotic BCL-2 family member [12].

An important issue arises as to whether 4-1BB signaling induces qualitative changes in CD8+ T cells, such as enhanced cytotoxic activity, in addition to the quantitative changes exemplified by clonal expansion and survival. The study by Cooper *et al.* indicates that 4-1BB signals modulate differentiation of naïve CD8+ T cells into interferon-γ-producing type 1 cytotoxic T cells (Tc1), without affecting the cytotoxic capacity of individual cells [13]. These results and others ([14] and references therein) indicate that 4-1BB has a crucial role in the generation of effector and memory cytotoxic T cells (CTLs) by increasing the number of CTLs and their survival and in the development of some effector functions that rely on cytokines.

Possible roles for 4-1BB in immunologic disorders

The finding that the level of 4-1BB expression in CD4+ T cells is similar to that in CD8+ T cells raises the possibility of a distinct role for 4-1BB in CD4+ T-cell function. In fact, studies that block or stimulate the 4-1BB costimulatory pathway demonstrate the involvement of 4-1BB in a variety of CD4+ T cell-mediated responses *in vivo*, including induction of Th cell anergy [15], an alloimmune response [16], an acute inflammation [17], an autoimmune disease [18] and a type 1 T helper (Th1) cell response to tumor cells ([19] and references therein). These findings are particularly noteworthy in that a crucial role for 4-1BB in the Th1-mediated immune response suggests that intervening in the 4-1BB costimulatory pathway could provide an immunotherapeutic approach to the treatment of inflammatory diseases.

The mechanism by which 4-1BB regulates CD4+ T cell-mediated responses remains to be defined, but as with CD8+ T cells, signaling through 4-1BB appears to promote cell proliferation and survival *in vitro* [20,21]. However, *in vivo* observations by Sun *et al.* indicate that the mechanism of 4-1BB costimulation in CD4+ T cells is different from that in CD8+ T cells [18]. Although costimulatory signaling via 4-1BB induces a clonal expansion of CD4+ T cells shortly after immunization, the activated CD4+ T cells are rapidly cleared via activation-induced cell death (AICD). The difference might be the result of intrinsic properties of the two T-cell subsets in terms of their proliferative and apoptotic responses to TCR [22] or TCR/4-1BB signals. At this point however, further studies are needed before it can be concluded that 4-1BB signals differentially affect CD4+ and CD8+ T-cell responses.

Another possible mechanism for 4-1BB regulation of CD4+ T-cell responses might be via 4-1BB-signal augmentation of the immunosuppressive activity of CD4+CD25+ regulatory T cells, which results in suppression of CD4+ T-cell-mediated immune responses (both Th1

and Th2). This intriguing idea is based on two *in vivo* observations: (1) agonistic anti-4-1BB monoclonal antibodies (mAbs) have been shown to abrogate T-cell-dependent antibody responses, but not T-cell-independent antibody responses, via the induction of helper T-cell anergy [15] and (2) agonistic anti-4-1BB mAbs have been shown to ameliorate experimental autoimmune encephalomyelitis (EAE) [18]. These two observations, coupled with the fact that 4-1BB is preferentially expressed in $CD4^+CD25^+$ regulatory T cells compared with non-regulatory $CD4^+$ T cells [3,4], are certain to lead to further studies to clarify this aspect of the regulatory process.

To date, four possible therapeutic strategies involving intervention in the 4-1BB costimulatory pathway have been published. First, stimulating 4-1BB using potent agonistic anti-4-1BB antibody was shown to effectively eradicate established tumors ([19] and references therein). Second, blockade of the 4-1BB costimulatory pathway using an anti-4-1BBL antibody was demonstrated to ameliorate virus-induced myocarditis [17]; this approach might also have some potential against autoimmune diseases and could prolong allograft survival. Third, it was suggested that amelioration of the autoimmune disease EAE by agonistic anti-4-1BB antibody was the result of the induction of apoptosis in autoreactive T cells [18]; it was assumed that the autoreactive T cells were in an activated state and expressed 4-1BB on the cell surface, which allowed selective induction of apoptosis in these cells by the agonistic anti-4-1BB antibody, much like the depletion of autoreactive T cells by cytotoxic antibodies in other contexts. Finally, the abrogation of humoral immune responses by agonistic anti-4-1BB antibody reported by Mittler *et al*. [15] could be useful in the treatment of autoimmune diseases such as systemic lupus erythematosus (SLE).

Concluding remarks

The expression pattern of 4-1BB suggests that this receptor might be involved in the innate immune response (e.g. by promoting activation and survival of neutrophils and macrophages) as well as the adaptive immune response. 4-1BB might also have an important role in the induction phase of immune responses by providing DCs with an activation signal, which in turn leads to activation of T cells via the 4-1BB–4-1BBL axis. Current evidence suggests that signaling via 4-1BB can elicit both Th1-mediated and $CD8^+$ T cell-mediated immune responses. It remains to be determined whether 4-1BB is involved in Th2-mediated immune responses or in the effector phase of immune responses. Clinical studies offer the possibility of using blockade or stimulation of the 4-1BB costimulatory pathway as a novel immunotherapeutic approach to the treatment of immunological disorders.

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