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MINI REVIEW

4-1BB signaling beyond T cells

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Originally discovered as a T cell-activating molecule, 4-1BB (CD137) is now also recognized as an activator of non-T cells, thus imparting a new dimension to its potential *in vivo* effects. 4-1BB expression is seen on a variety of non-T cells including activated dendritic cells (DCs), monocytes, neutrophils, B cells and natural killer (NK) cells, and promotes their individual effector functions. The T cell- and non-T cell-activating ability of 4-1BB may be the basis of its powerful anti-cancer, anti-autoimmune and anti-viral effects. Here we discuss the consequence and importance of 4-1BB signaling in non-T cells. We consider its effects on immune regulation, and the distinct and/or overlapping pathways involved in these responses, as well as possible therapeutic applications.
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INTRODUCTION

4-1BB (CD137; TNFRSF9), discovered originally on T cells,[1] is a 50- to 55-kDa protein concerned with progressive immunity.[2,3] 4-1BB expression is in most cases activation induced. Exceptions are dendritic cells (DCs) and Foxp3$^+$ regulatory T cells which express this antigen in a constitutive manner.[4,5] The *in vitro* effects of 4-1BB are varied and involve cell cycle progression in lymphocytes, cytokine induction, prevention of activation-induced cell death and induction of anti-apoptotic genes.[6] There is a disparity between its *in vitro* and *in vivo* effects. While it supports the activation of both CD4$^+$ and CD8$^+$ T cells *in vitro*, it induces a response skewed towards CD8$^+$ T cells *in vivo* and kills CD4$^+$ T cells or inactivates their effector functions.[7] Much progress has been made in understanding the basis of the biased *in vivo* 4-1BB effects, and several causative molecules have been indentified, the main players being interferon (IFN)-γ,[8–10] tumor necrosis factor (TNF)-α,[8] transforming growth factor-β,[11,12] and indoleamine 2,3-dioxygenase.[13,14]

Nearly a decade after the discovery of 4-1BB, two groups demonstrated functional expression of 4-1BB on DCs,[4,15] an attribute somewhat conflicting with its proposed role as a T cell-activating molecule.[2,3] In the years that followed, functional expression of 4-1BB was noted on several non-T cells including monocytes, neutrophils, macrophages, B cells and natural killer (NK) cells (see below). The identification of functional 4-1BB expression on non-T cells is important not only for understanding the *in vivo* effects of 4-1BB but also for helping to design effective therapeutic strategies against a variety of T cell- as well as non-T cell-mediated immune diseases. The effects of anti-4-1BB antibody (Ab) on T cells have been extensively investigated and unifying theories have been proposed as to their mechanistic basis. A comprehensive review dealing with the various effects of anti-4-1BB on non-T cells, however, is lacking. In this review we will focus on the functions of 4-1BB in non-T cells including DCs, monocytes, neutrophils, B cells and NK cells, and discuss how its expression might be manipulated to treat various immune diseases.

EFFECTS OF 4-1BB CROSSLINKING IN NON-T CELLS

Dendritic cells

DCs are potent antigen-presenting cells that possess the ability to stimulate naive T cells.[16] Besides presenting antigens to T cells, activated DCs secrete a variety of immune modulators that have manifold functions in health and disease.[17] Of all the non-T cells studied to date, the function of 4-1BB has been most extensively studied in DCs. Futugawa *et al.*[15] were the first to show that DCs express 4-1BB constitutively and that signaling *via* anti-CD40 downregulates this expression. These authors suggested that signaling *via* 4-1BB using 4-1BB ligand (4-1BBL)-transfected cells upregulates B7-1 and B7-2, and increases IL-6 and IL-12 secretion by DCs.[15] That 4-1BB transmits activation signals through 4-1BB in DCs was demonstrated by the absence of IL-12 production in response to anti-CD40 when the 4-1BB/4-1BBL interaction was blocked by anti-4-1BBL.[15] Confirmation that DCs express functional 4-1BB came from Wilcox *et al.*[4] These authors observed that administration of *in vivo* agonistic anti-4-1BB monoclonal antibody (mAb) to naive mice enhanced the ability of DCs to stimulate *in vitro* T cell-proliferative responses to both alloantigens and nominal antigens.[4] In addition, Zhang *et al.*[18] underscored the importance of 4-1BB signaling in DCs during viral infection; they showed that *in vitro* activation of DCs by anti-4-1BB led to phosphorylation of STAT3, which in turn strengthened CD8$^+$ T-cell responses augmenting viral clearance, and this effect was corroborated using STAT3 conditional knockout mice.[18] Interestingly, 4-1BB$^{-/-}$ mice have increased frequencies of DCs,[19] but these DCs have decreased survival rates,[20] highlighting the importance of 4-1BB in

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DC regulation. Taken together, these findings show that 4-1BB ligation by agonistic anti-4-1BB on DCs has important effects.

Monocytes

Monocytes are bone marrow-derived immature macrophages. They have a distinct phenotype, and upon maturation are involved in killing bacteria and tumors by producing cytokines and other immune modulators.[21] In 1995, Schwarz *et al.*[22] observed that 4-1BB is induced in human blood monocytes by IL-1β, lipopolysaccharide and phorbol myristate acetate. Similar findings were reported by Kienzle *et al.*[23] Activation of primary monocytes by immobilized agonistic anti-4-1BB mAb induced the production of IL-8 and TNF-α, but caused down-modulation of IL-10.[23] Interestingly, crosslinking of 4-1BB on monocytes causes deletion of B cells.[23] Further analysis revealed that deletion of B cells in these experiments requires cell contact with monocytes, as well perhaps as products secreted by the latter.[23]

B cells

B lymphocytes play critical roles in immune regulation.[24] Besides secreting immunoglobulins (Igs) and Abs, they are important for priming T-cell responses.[25] In addition to their neutralizing effect on viruses,[26] Abs produced by autoreactive B lymphocytes contribute to disease severity in autoimmune conditions such as lupus and rheumatoid arthritis.[27] 4-1BB transcripts were observed more than a decade ago in human B cells stimulated by phorbol myristate acetate and anti-Ig Abs,[22] but possible functional consequence were not explored. Recently, Zhang *et al.*[28] demonstrated that 4-1BB was expressed by human B cells when total peripheral blood monocytic cells were activated with pokeweed mitogen but not when purified B cells were used. 4-1BB expression on human B cells was also unregulated by anti-CD40/anti-Ig, and in combination with IFN-γ, but not when IL-2, -6 and -15, and TNF-α were used.[28] Interestingly, addition of either IL-4, IL-10 or IL-21 to B cells in the presence of anti-CD40/anti-Ig caused downregulation of 4-1BB expression.[28] When P815 cells, transfected to express 4-1BBL, were co-incubated with human B cells, they increased their survival, enhanced their proliferation and unregulated TNF-α and -β, suggesting that 4-1BB expression by B cells is functional. The identification of 4-1BB$^+$ B cells in the germinal centers of tonsils indicates a possible role in humoral immunity.[28] Our laboratory has also observed that murine B cells proliferate in

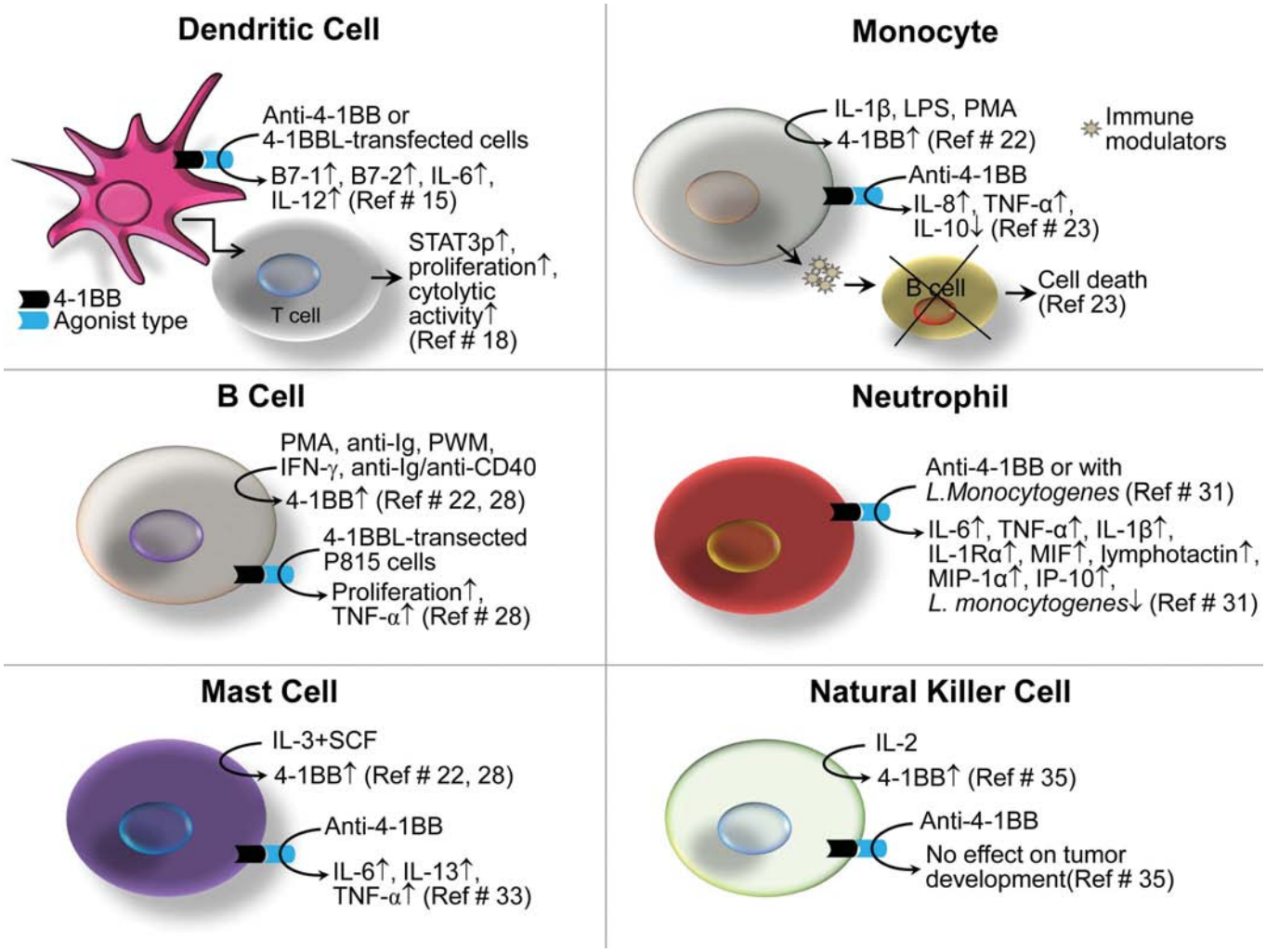

Figure 1 Consequence of 4-1BB receptor ligation in non-T cells. 4-1BB, a powerful T cell-specific costimulatory molecule, is also expressed by a number of non-T cells. The expression of 4-1BB is activation dependent with the exception of DCs and neutrophils who express this antigen in a constitutive fashion. Ligation of 4-1BB either by agonist anti-4-1BB or 4-1BBL-transfected cells relay activation signals resulting in cellular proliferation, cytokine induction, bactericidal activity, and support T-cell effector functions. DC, dendritic cell; 4-1BBL, 4-1BB ligand.

the presence of anti-4-1BB and anti-μ Abs and show enhanced production of type I IFNs but there is no effect on Ig class switching either *in vitro* or *in vivo* (unpublished observations). Nevertheless, the identification of functional 4-1BB on B cells is an important finding and perhaps explains why B-cell numbers/function are affected in mice treated with anti-4-1BB alone or in combination with autoimmune-inducing proteins.[8]

Neutrophils

Neutrophils, components of the innate immune system, are produced in huge numbers in response to infection, trauma and inflammation, and form an early line of defense against bacterial and parasitic infections by releasing immune modulators and various cytokines.[29,30] Murine neutrophils constitutively express 4-1BB.[31] *In vitro* stimulation of purified neutrophils with either anti-4-1BB alone or in combination with heat-killed *Listeria monocytogenes* enhanced production of IL-6 and TNF-α as well as IL-1β, IL-1Rα, migration inhibitory factor, lymphotactin, macrophage inflammatory protein-1α, IFN-γ-inducible protein-10 and thymus-derived chemotactic agent.[31] Additionally, activation of neutrophils by anti-4-1BB *in vitro* decreased the burden of *L. monocytogenes*, and increased the phagocytic ability of neutrophils while decreasing associated pathology,[31] underscoring the importance of 4-1BB signaling in these cells.

Mast cells

Mast cells are important immune effectors and play critical roles in hypersensitivity and allergic reactions.[32] 4-1BB is expressed by mast cells and is increased further upon activation with IL-3 and stem cell factor.[33] Incubation of activated but not resting mast cells with agonistic anti-4-1BB induces production and secretion of IL-6, IL-13 and TNF-α.[33] Although 4-1BB signaling activates mast cells and induces cytokine production, it does not influence mast cell proliferation.[33] Also, the turnover rate of mast cells from bone marrow precursors is comparable in 4-1BB$^{+/+}$ and 4-1BB$^{-/-}$ mice, indicating that 4-1BB is not required for mast cell differentiation.

NK cells

NK cells are large, bone marrow-derived, granular lymphocytes that lyse tumor cells and virus-infected cells, bypassing major histocompatibility complex restriction and prior sensitization.[34] 4-1BB expression was noted on activated but not resting NK cells.[35] Although 4-1BB is expressed by NK cells, ligation of 4-1BB on the cells by agonistic 4-1BB during tumor progression did not influence the cytolytic function of the NK cells or tumor activity, suggesting that the anti-tumor activity of anti-4-1BB mAb does not control the cytolytic components of NK cells.[35] Interestingly, *in vivo* anti-4-1BB mAb treatment kills NK cells, presumably *via* increased induction of IFN-γ.[8] Consistent with the importance of 4-1BB in NK cell regulation, 4-1BB$^{-/-}$ mice have reduced NK cell numbers.[36]

CONCLUSION

As is evident from the preceding sections, ligation of 4-1BB on non-T cells has important functional consequences (Figure 1). Since anti-4-1BB therapy has multiple targets in the form of T as well as non-T cells, targeting these cells with anti-4-1BB could have profound therapeutic effects. Future studies should address *in vivo* targeting of non-T cells by agonistic 4-1BB mAbs, especially in clinical conditions where T cells have a minimal role in disease progression. Such studies hold promise of providing useful platforms for designing effective treatments for a number of such diseases.

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