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BRIEF REVIEWS

2B4/CD48-Mediated Regulation of Lymphocyte Activation and Function¹Erika Assarsson,^{2*} Taku Kambayashi,[‡] Catrine M. Persson,^{*} Benedict J. Chambers,^{*} and Hans-Gustaf Ljunggren^{2*}

2B4 (CD244) is a member of the CD2 subset of the Ig superfamily. This molecule is expressed on innate immune cells, including NK cells, and on subsets of T cells. The 2B4 molecule interacts with CD48, which is widely expressed on hemopoietic cells. Although earlier reports demonstrated a role for 2B4 as an activating receptor in both mice and humans, recent studies of 2B4-deficient mice have suggested that 2B4 functions predominantly as an inhibitory receptor in mice. In addition, 2B4 may also act as a costimulatory ligand for cells expressing CD48. Thus, the 2B4 molecule is more multifunctional than previously understood. In this study, we delineate the current view of 2B4-CD48 interactions among lymphocytes and other cells. The Journal of Immunology, 2005, 175: 2045–2049.

Numerous receptor/ligand pairs are important in the cross-talk among cells of the immune system (1–3). One group of such molecules is the CD2 subset of the Ig superfamily, which includes CD2, CD2F-10, CD48, CD58, CD84, CD150 (signaling lymphocytic activation molecule), CD229 (Ly9), CD244 (2B4), B lymphocyte activator macrophage expressed, CS1, and NK-T-B Ag (NTB-A) (4). These molecules commonly bind to each other (homotypic adhesion) or to other members of the same family (heterotypic adhesion). The functions of many of these molecules have been described elsewhere (5–8). The present review focuses on new data providing information on the functional outcome of interactions between 2B4 and CD48, with implications for lymphocyte-lymphocyte interactions.

Expression of 2B4 and its interaction with CD48

2B4 is expressed on many cells belonging to the innate arm of immunity. In mice, 2B4 is found on all NK cells, subsets of $\gamma\delta^+$ T cells, monocytes, mast cells, as well as on a subset of memory-like CD8⁺ T cells (6, 9, 10). In humans, 2B4 is expressed on NK cells and $\gamma\delta^+$ T cells, ~50% of CD8⁺ T cells, and on subsets of ba-

sophils, monocytes, and eosinophils (11–15). In addition, T cells have been shown to acquire 2B4 expression under certain activating conditions. For instance, murine CD8⁺ T cells acquire 2B4 expression upon cytokine stimulation in vitro and during viral infection in vivo (10). The fraction of 2B4⁺CD8⁺ T cells increases in HIV-positive patients with the progression of disease (16). 2B4 is also found on a large proportion of effector/memory CD4⁺ T cells in CMV-infected individuals (17).

In both mice and humans, 2B4 binds to CD48 (18, 19), which is broadly expressed on hemopoietic cells (20–23). At least in the mouse, 2B4 does not appear to bind any other molecule than CD48 (24). CD48 expression is up-regulated upon viral infection and by stimulation of IFN- $\alpha\beta$ and IFN- γ (6, 23).

Activating and inhibitory functions of the 2B4 receptor

Murine NK cells and T cells express two isoforms of 2B4, 2B4 short (2B4S) and 2B4 long (2B4L), derived from alternative mRNA splicing of its cytoplasmic domains (25, 26). The cytoplasmic tail of 2B4L contains seven potential tyrosine phosphorylation sites, five of which are unique to 2B4L (25, 26). Four of these sites resemble immunoreceptor tyrosine-based switch motifs (ITSM).³ 2B4S contains only one ITSM (the one most adjacent to the membrane), suggesting that the two receptors signal differently. In fact, 2B4S and 2B4L have been reported to be activating and inhibitory, respectively, when expressed in the rat NK cell line RNK-16 (26).

Initial studies suggested that 2B4 was an activating receptor on murine NK and T cells (6). Ab-mediated stimulation of 2B4 enhanced lytic activity and IFN- γ production by NK cells in vitro. The recent generation of 2B4-deficient mice (27) has allowed more direct studies on the functional role of 2B4 on murine NK cells both in vitro and in vivo. Surprisingly, 2B4-deficient NK cells displayed enhanced cytotoxicity against CD48-expressing cells in vitro (27, 28). Furthermore, in the absence of functional 2B4-CD48 interactions between the NK cell and the target cell, the IFN- γ production by NK cells was

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³ Abbreviations used in this paper: ITSM, immunoreceptor tyrosine-based switch motif; EAT-2, EWS/Flil-activated transcript 2; LAT, linker for activation of T cells; SAP, signaling lymphocytic activation molecule-associated protein.

enhanced. Finally, 2B4-deficient mice eliminated CD48-expressing tumor cells more efficiently than wild-type mice (28). These results suggest that 2B4 acts as an inhibitory molecule on murine NK cells. The discrepancy between these and the earlier findings is not clear. However, one possible explanation is that the anti-2B4 Ab used in the initial experiments blocked an inhibitory interaction between 2B4 and CD48 rather than cross-linking 2B4 on the NK cells (28). Further support for an inhibitory function of 2B4 has come from the observation that 2B4 accumulation at the interphase between NK cells and CD48-expressing cells correlates inversely with the ability of the NK cells to lyse the latter cells (29). The inhibitory receptor function of 2B4 is interesting per se, as it reveals an MHC-independent mechanism of NK cell inhibition (27, 28, 30).

The human *2B4* gene encodes a transcript that is closely related to murine 2B4L (13, 31) as well as a splice variant that differs in the extracellular domains (32). Several studies have demonstrated an activating role for 2B4 on human NK cells. Anti-2B4-mediated stimulation of NK cells initiates polyphosphoinositol turnover and leads to increased intracellular Ca^{2+} levels, enhanced cytolytic function, and cytokine production (6). 2B4 can also act as a costimulatory receptor on human NK cells, enhancing signals from other NK cell receptors under limited ITAM-mediated activation (33). With respect to induction of cytotoxicity, it is still not entirely clear whether 2B4 provides costimulatory signals in the context of other receptor-ligand interactions or whether 2B4 is capable of triggering cytotoxicity independently of other receptor signals. In contrast to the observations above, at the early stages during human NK cell differentiation, 2B4 seems to function as an inhibitory receptor (34). It has been suggested that negative 2B4 signaling might prevent immature NK cells from killing normal autologous cells and thereby ensuring self-tolerance.

Signal transduction through 2B4

The mechanisms through which 2B4 signals are still under investigation. NK cell activation through 2B4 is accompanied by phosphorylation of tyrosine-based motifs in its cytoplasmic tail and recruitment of the signaling lymphocytic activation molecule-associated protein (SAP) and the Src family kinase Fyn (35, 36). SAP is a small Src homology 2 domain-containing protein expressed in NK and T cells (37). X-linked lymphoproliferative disease is a severe immune deficiency characterized by an inability to control EBV infection. X-linked lymphoproliferative patients have a mutated *SAP* gene (37). As a result, the SAP protein is either absent or dysfunctional. In these patients, cross-linking of 2B4 does not only fail to transduce triggering signals (38–40), but may even mediate inhibition of NK cell cytotoxicity (41). This suggests that SAP is essential for the activating function of 2B4. Cross-linking leads to recruitment of 2B4 to lipid rafts essential for tyrosine phosphorylation (42). The phosphorylated ITSMs of 2B4 have different functions. Whereas all four phosphorylated ITSMs can bind to SAP, the third ITSM can also recruit inhibitory signaling molecules including Src homology region 2 domain-containing phosphatase 1, Src homology region 2 domain-containing phosphatase 2, SHIP, and Csk (36). It has been shown that SAP cannot bind to 2B4 at the same time as either of these molecules (36). Therefore, SAP may block inhibition of NK cells by preventing negative signaling molecules from binding 2B4. It has also been shown that SAP can recruit Fyn to the cytoplasmic ITSM of

2B4 (35, 36). Fyn can in turn induce further ITSM phosphorylation and subsequent recruitment of downstream effector molecules and activation of the NK cell (35, 36). Thus, the presence or absence of SAP in the cell may affect the 2B4 receptor function. In this regard, it is interesting that viral infections can up-regulate SAP expression in mice (43). SAP is closely related to EWS/Fli1-activated transcript 2 (EAT-2). Similar to SAP, EAT-2 encodes a free Src homology 2 domain that binds a specific tyrosine motif in the cytoplasmic tail of some receptors of the CD2 subset of the Ig superfamily (44). In contrast to SAP, little is known about the biological role of EAT-2 in association with 2B4 (8). 2B4 and CD48 are both located to lipid rafts, also called glycolipid-enriched microdomains (45). In the lipid rafts, 2B4 associates with the linker for the activation of T cells (LAT). In the absence of LAT, 2B4-mediated signaling is impaired (45). LAT may thus be an important intermediate in 2B4-mediated signaling.

T cells costimulate each other through 2B4-CD48 interactions

It is well established that T cells are regulated by signals generated from interactions with other cells, e.g., APC, epithelial cells, and stromal cells, involving the TCR, costimulatory receptors, and adhesion molecules. Some reports also show that interactions taking place between individual T cells are critical for certain T cell responses. For example, it has been suggested that B7-expressing T cell clones can stimulate each other through interactions with CD28 (46). Furthermore, CD40-CD40 ligand interactions between $CD4^{+}$ and $CD8^{+}$ T cells have been demonstrated to be important for memory formation in some model systems (47).

We have shown that 2B4-expressing activated T cells, or tumor cells transfected with 2B4, enhance the proliferation of CD48-expressing T cells stimulated with specific Ag or IL-2 (10, 24). Blocking 2B4 on the stimulatory cells or CD48 on the responding T cells reduced proliferation. Furthermore, when naive T cells were stimulated with anti-CD3 Ab in short-term assays, the number of CD69-expressing cells increased in the presence of 2B4-expressing cells. These findings led us to speculate that 2B4 expressed on activated $CD8^{+}$ T cells may act as a costimulatory ligand for CD48 on neighboring T cells, enhancing proliferation and other effector functions of the latter.

In relation to this, a recent study nicely demonstrated that transduction of TCR-transgenic T cells with either 2B4S or 2B4L increased the cytotoxic activity against both $CD48^{-}$ and $CD48^{+}$ peptide-pulsed target cells (48). These results show that 2B4-CD48 interactions take place between the T cells and improve T cell cytotoxicity independently of CD48 expression on the target cells. The increased cytotoxicity is efficiently blocked when the anti-CD48 Ab was present both during the transduction and during the cytotoxicity assay (48). In this system, it was speculated that signals were transduced downstream of 2B4. However, an alternative explanation for the results above is that 2B4 acted as a ligand for CD48, accounting for some of the phenomena observed. This alternative interpretation is supported by studies showing that 2B4-transfected tumor cells enhance activation and proliferation of T cells that express CD48 (24). In line with this, immobilized anti-CD48 Ab increases CD40-mediated activation of human B cells, adding further support to the notion of a costimulatory role for CD48 (49). It shall be pointed out, however, that none of the

above-discussed interpretations can be excluded on the basis of the results discussed above.

2B4 and CD48 both reside in glycolipid-enriched microdomains, which contain many other molecules involved in signaling (45). Therefore, it is also possible that 2B4 and CD48 binding leads to adhesion and therefore prolongs cellular interaction and facilitates signaling by other molecules.

How CD48 transmits signals is not clear. Although CD48 is a GPI-anchored molecule and lacks intracytoplasmic domains, it can physically associate with G proteins and to members of the Src family of tyrosine kinases in lipid rafts (50). For example, Lck has been reported to associate with CD48 (51, 52). A recent report has also described a complex formation among the IL-18R α , IL-18, and CD48 that in turn binds the IL-18R β . This complex has been implicated in delivering the IL-18 signal (53). If the latter finding has any relation to the present discussion on 2B4-mediated interactions with CD48 remains to be investigated.

NK cells costimulate each other through 2B4-CD48 interactions

The studies above suggest an important role for interactions between 2B4 and CD48 in the course of T cell activation and proliferation. Recent data suggest that similar interactions may take place between NK cells. We have demonstrated that IL-2-induced proliferation of both murine and human NK cells is reduced when either anti-2B4 or anti-CD48 Abs are added to the cultures (24). These results support findings by Valiante and Trinchieri (11), who have shown that human NK cells proliferate poorly in response to IL-2 and IL-12 in the presence of anti-2B4 Ab. A recent study has extended these two observations. Using gene-deficient mice, Lee et al. (54) found that 2B4-CD48 interactions are essential for IL-2-driven expansion and activation of murine NK cells. In the absence of a functional 2B4-CD48 interaction between NK cells, cytotoxicity and IFN- γ secretion upon tumor target exposure were severely impaired (54). However, it is still not entirely clear from any of the above-discussed experiments whether 2B4 acts exclusively as a receptor on the NK cells or whether 2B4 also acts as a ligand that costimulates other NK cells through interaction with CD48 (compare discussion above regarding 2B4-CD48 interactions in T cell-T cell interactions). Noteworthy, the expression of 2B4 is up-regulated on NK cells upon activation with poly(I:C) in vivo (55). Thus, 2B4-CD48 interactions as well as interactions between other members of the CD2 subset of the Ig superfamily could be important for rapid expansion of activated NK cells at sites of infection and/or inflammation.

NK cells costimulate T cells through 2B4-CD48 interactions

NK cells produce a number of cytokines, including IFN- γ , TNF- α , IL-13, and GM-CSF. These cytokines provide important immunoregulatory properties through which NK cells can influence the outcome of adaptive immune responses (56). NK cell depletion in mice impair CTL responses in different models of infection and cancer as well as the development of adaptive immune responses in experimentally induced autoimmunity and hypersensitivity reactions (57, 58). Although cytokine production by NK cells clearly influences T and B cell responses, it has until recently been unclear whether NK cells affect adaptive immune cell functions through direct cellular interactions.

In a recent study, it was demonstrated that activated NK cells significantly increases the proliferation of anti-CD3-stimulated

CD8⁺ and CD4⁺ T cells (24). This required direct physical contact between the NK cells and the T cells. The proliferation of T cells was reduced to background levels upon blocking of 2B4 or CD48. These findings argue for a scenario in which NK cell-mediated enhancement of T cell proliferation involves physical contact, although additional contribution of secreted factors cannot be excluded. The addition of NK cells to anti-CD3-stimulated CD8⁺ T cells also enhances CD69 expression on the CD8⁺ T cells, and this phenomenon was dependent on 2B4-CD48 interactions (24). These findings suggest a role for 2B4 and CD48 in interactions between NK cells and T cells.

Conclusions

Altogether, the studies reviewed in this article suggest that interactions between 2B4 and CD48 may have at least three different outcomes in responding lymphocytes (Fig. 1). On different subsets of lymphocytes, the 2B4 molecule may function either as a stimulatory/costimulatory receptor or as an inhibitory receptor upon interaction with CD48-expressing cells. In parallel, the 2B4 molecule may function as a costimulatory ligand upon interaction with CD48-expressing cells. If the reasoning above holds true, 2B4 now joins a growing list of molecules that have a bidirectional function, i.e., transducing stimulatory/costimulatory or inhibitory signals into the expressing cell and, in addition, acting as a ligand to stimulate/costimulate CD48-expressing cells. In addition, the signals above may operate simultaneously. Less exciting is the possibility that 2B4-CD48 interaction merely leads to adhesion, which facilitates signaling through other receptor/ligand pairs. Although the focus of this review has been on 2B4-CD48 interactions among NK cells and T cells, quite possibly these interactions may also occur between other sets of lymphocytes, such as B cells and NKT cells. The findings should open up new areas

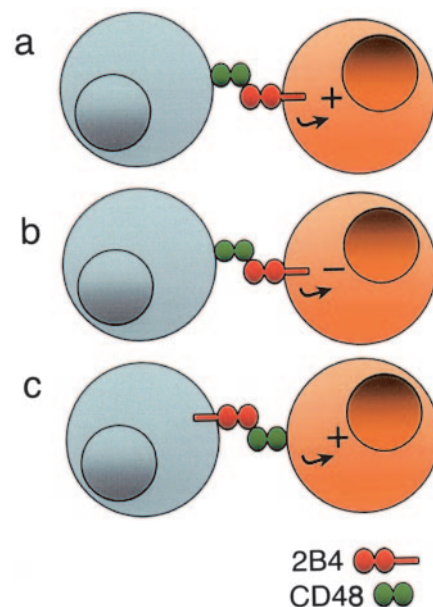


FIGURE 1. Principles of 2B4-CD48 interactions. *a*, 2B4 acts as a stimulatory/costimulatory receptor upon interactions with CD48 on the neighboring cell. *b*, 2B4 acts as an inhibitory receptor upon interactions with CD48 on the neighboring cell. *c*, CD48 acts as a stimulatory/costimulatory receptor upon interacting with 2B4 on the neighboring cell. 2B4 (red, transmembrane), CD48 (green, GPI linked). +, Stimulation/costimulation; -, inhibition.

of research, including studies on the role of these interactions in infectious disease and tumor models.

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