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CUTTING EDGE

Cutting Edge: The NK Cell Receptor 2B4 Augments Antigen-Specific T Cell Cytotoxicity Through CD48 Ligation on Neighboring T Cells[1]

Kyung-Mi Lee,[2] Sadhna Bhawan,* Takashi Majima,* Hairong Wei,* Michael I. Nishimura,† Hideo Yagita,‡ and Vinay Kumar**

2B4 is expressed on all NK and a subset of memory/effector CD8$^+$ T cells. 2B4 binds to CD48 and activates NK cytotoxicity, but its function on CD8$^+$ T cells is not clear. Furthermore, two isoforms of 2B4 (2B4S and 2B4L) exist in mice but the role of individual isoforms is not known. To address these questions, we generated primary T cell cultures from L^d-specific 2C/Rag2$^{-/-}$ TCR transgenic mice and transduced them with 2B4S or 2B4L. 2B4S- or 2B4L-transduced T cells showed greater cytotoxicity over control cells against CD48$^+$ and CD48$^-$ targets, suggesting that ligation of 2B4 by CD48 on target cells was not necessary for 2B4 function. Rather, 2B4/CD48 interaction on adjacent T cells appeared to be critical for cytotoxicity. Therefore, 2B4 functions as a costimulator of CD8$^+$ T cells in MHC-restricted cytotoxicity. We conclude that 2B4/CD48 interactions among T cells themselves can augment CTL lysis of their specific targets. The Journal of Immunology,* 2003, 170: 4881–4885.

Belonging to the Ig superfamily, 2B4 is a 66-kDa membrane protein. 2B4 is closely related to CD2 subfamily members, such as signaling lymphocyte activation molecule (SLAM),[3] CD48, CD58, CD84, and Ly9, and shares its ligand CD48 with CD2 (1, 2). It is expressed on all NK cells, $\gamma\delta$ T cells, monocytes, basophils, and a subset of CD8$^+$ $\alpha\beta$ T cells (1, 2). Most of the information available on 2B4 comes from studies of human NK cells. Upon ligation with CD48, 2B4 stimulates cytotoxicity, IFN-γ secretion, and granule exocytosis (2, 3). Although human NK cells express one 2B4 gene product, murine NK cells express two isoforms of 2B4 (2B4S and 2B4L) resulting from alternative splicing (4). Previous studies have demonstrated an opposing function of 2B4S and 2B4L, where 2B4S activated target cell lysis while 2B4L inhibited the lysis (4).

Approximately 1–2% of all splenic CD8$^+$ T cells from normal mice express 2B4, and this subset of T cells displays a memory/effector phenotype (5). Upon influenza virus infection, 2B4$^+$ T cells increased up to 5–10% and this is frequently accompanied with acquisition of NK-activating receptors such as NK1.1 (5). It has been shown that murine 2B4$^+$CD8$^+$ T cells cultured in high dose of IL-2 acquired non-MHC-mediated killing activity against tumor targets, suggesting that 2B4 on T cells can also function to stimulate non-MHC-restricted lysis (2). However, anti-2B4 mAb cross-linking on human CD8$^+$ T cells did not trigger redirected lysis of FcR-bearing targets, cytokine production, or proliferation (3). The contradictory results from these two experiments could be due to the difference in 2B4 signaling between human and murine NK cells. Alternatively, 2B4 might function as a coreceptor for activating receptors that might have been acquired during IL-2 exposure of murine T cells. To test this hypothesis, it was essential to have a clonal population of T cells presenting a defined TCR which also coexpresses 2B4. Therefore, we generated Ag-primed 2C/Rag2$^{-/-}$ transgenic T cells that recognize L^d class I molecules and can lyse L^{d+} targets. Primed 2C T cells did not express 2B4, thus we transduced them with either the long or short form of 2B4 to analyze their role in mediating non-MHC-restricted lysis and costimulation of MHC-restricted lysis. Our data show that neither the short nor long form of 2B4 could induce non-MHC-restricted lysis by CD8$^+$ T cells, but rather functioned as a costimulatory receptor in these cells. We also found that this costimulation was dependent on binding to CD48 expressed on the adjacent T cells, but not on the target cells. Thus, 2B4 may represent a novel T cell costimulator that enhances MHC-restricted cytolysis by interacting with CD48$^+$ neighboring T cells.

Materials and Methods

Mice

The 2C TCR transgenic mice were developed as described previously (6), and obtained from Dr. T. Gajewski (University of Chicago, Chicago, IL). The 2C (H-2^b) T cells are specific for either the alloantigen L^d complexed with the octapeptide p2Ca (LSPFPFDL) (7) or the syngeneic K^b molecule complexed with the synthetic peptide SIYRYYGL. These mice were intercrossed with Rag2-deficient mice to obtain 2C/Rag2$^{-/-}$ mice (8).

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[3] Abbreviations used in this paper: SLAM, signaling lymphocyte activation molecule; SAP, SLAM-associated protein.

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Cell lines and transfectants

CD48$^+$P815 mastocytoma cells were obtained from American Type Culture Collection (Manassas, VA). CD48$^-$P815 cells were obtained from Dr. J. Shatzle (University of Texas Southwestern Medical Center, Dallas, TX). CD48$^-$P815 cells transfected with murine B7-1 (P815.B71) were obtained from Dr. T. Gajewski (University of Chicago). A murine fibrosarcoma line, Ag104 (9), derived from C3H/HeN mice (H-2^K) and its stable transfectants expressing L^d, and the C57BL/6-derived MC57 fibrosarcoma cell line (10) and its stable transfectants expressing the 2C peptide SIYRYYGL fused to enhanced green fluorescent protein (11), were obtained from Dr. H. Schreiber (University of Chicago). A retroviral packaging cell line, Plat-E (12), was obtained from Dr. T. Kitamura (University of Tokyo, Tokyo, Japan). All cell lines were cultured in DMEM supplemented with 10% FCS and incubated at 37°C in 10% CO_2 atmosphere.

Abs and flow cytometry

PE-conjugated anti-CD48 mAb (clone HM48-1), anti-2B4 mAb (clone 2B4), hamster IgG group1 and mouse IgG2b, κ were purchased from BD PharMingen (San Diego, CA). Purified anti-CD48 mAbs (HM48-1) were obtained from Dr. H. Yagita (Juntendo University School of Medicine, Tokyo, Japan). Cells were suspended in PBS supplemented with 2% FBS, and then incubated with 20 μl of anti-CD16/32 culture supernatant (2.4G2) to block FcR before staining with PE-conjugated Abs for 30 min at 4°C.

Generation of in vitro-primed 2C/Rag2$^{-/-}$ T cells

2C/Rag2$^{-/-}$ transgenic T cells (H-2^b) were harvested from lymph nodes. Whole lymph node cells (0.2×10^6) were cocultured with 1×10^6 mitomycin C-treated CD48$^-$P815.B71 cells (H2-d) for 5 days in 24-well tissue culture plates (passage P1, priming). At day 5, live cells were Ficolled and restimulated with mitomycin C-treated P815.B71 cells for additional 5 days (passage 2, P2). After the third round of stimulation (P3), these T cells developed maximum cytotoxicity against L^{d+} targets (data not shown). Therefore, the retroviral transduction and cytolytic assays were conducted using P3 cells.

Production of retroviral vectors and retroviral transduction

Murine 2B4L and 2B4S cDNAs inserted in pME18S mammalian expression vector (kindly provided by Dr. J. Schatzle, University of Texas Southwestern Medical Center) were digested with *Xho*I and *Not*I, and inserted into the sites present in the pg1 SAMEN CMV/SRα vector (13). Infectious retroviral supernatants were generated by transient transfection of the Plat-E ecotropic packaging cell line (12) using the calcium phosphate precipitation method (14). Culture supernatants were collected at 48 and 72 h following transfection, and their aliquots were stored at −80°C. On the day of transduction, retroviral supernatants were thawed and spun at 3000 rpm for 10 min to remove any debris.

Transduction of 2C/Rag2$^{-/-}$ T cells was performed with P3 T cells cocultured with P815.B71 for 2 days in 24-well plates. The culture medium was replaced with 1 ml of retroviral supernatant containing pg1 SAMEN vector or pg1 SAMEN vector containing 2B4S or 2B4L in the presence of 10 μg of polybrene. After addition of the retroviral supernatant, plates were centrifuged at 3000 rpm for 3 h at 4°C, and transferred to 37°C 10% CO_2 incubator. The next day, the retroviral supernatants were replaced with fresh retroviral supernatants, and this process was repeated. On the following day, transduced cells were harvested and live cells were collected.

^{51}Cr-release assay

Three thousand target cells labeled with ^{51}Cr as described (1) were incubated with transduced T cells for the time as indicated in the figure legends. Percent-specific lysis was determined as previously described (1).

Results

Retroviral transduction of primed 2C/Rag2$^{-/-}$ T cells with 2B4

To define the role of 2B4 on Ag-specific T cells, 2CRag2$^{-/-}$ transgenic CD8$^+$ T cells were primed in vitro. As shown in Fig. 1*A*, these T cells did not express any detectable level of 2B4, but almost all the cells expressed the 2B4 ligand CD48. Because they did not express 2B4, it provided a model system to test the role of each isoform of 2B4 by introducing the gene for 2B4S or 2B4L. Upon retroviral transduction, almost all the T cells expressed 2B4 as shown in Fig. 1*B*. In most cases, expression of 2B4L was higher than that of 2B4S, presumably due to its higher retroviral titer in the Plat-E culture supernatant. Next, we investigated the effector function of 2B4-transduced 2C T cells.

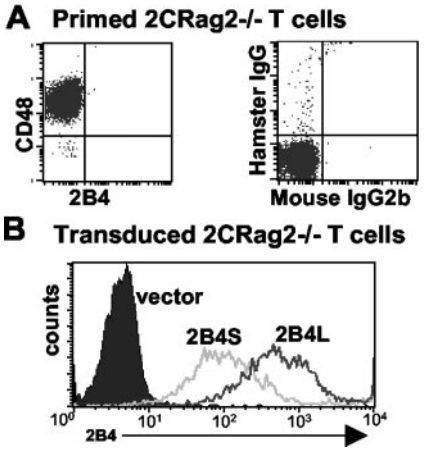

FIGURE 1. Retroviral transduction of 2C/Rag$^{-/-}$ T cells with 2B4. *A*, Passage 3 (P3) -primed 2C/Rag2$^{-/-}$ transgenic T cells generated from coculture with P815. B71 were gated on T cell blasts and analyzed by FACS for CD48 and 2B4 expression. Isotype controls for CD48 and 2B4, hamster IgG, and mouse IgG2b did not result in specific staining. *B*, P3 cells were retrovirally transduced with a plasmid (pg1 SAMEN) expressing 2B4S, 2B4L, or vector control, and live cells were collected the next day. Cells were stained with anti 2B4-PE and analyzed by FACS. In all cases ($n = 15$), 2B4 expression on the gated population was over 95%.

Expression of 2B4L or 2B4S augments the Ag-specific T cell cytotoxicity against CD48$^+$ targets as well as CD48$^-$ targets

Because 2B4 recognizes CD48 on the target cells, we chose a CD48-expressing P815 cell line (CD48$^+$P815) as the target for the cytotoxic assay. As shown in Fig. 2*A*, vector-transduced or nontransduced (data not shown) T cells showed highly specific lysis against CD48$^+$P815 targets. Expression of either 2B4S or 2B4L augmented the baseline CTL activity at all the ratios tested. Typically, cells expressing 2B4L showed higher killing than those expressing 2B4S. On a cell per cell basis, 2B4L-transduced T cells were at least three times and 2B4S-transduced T

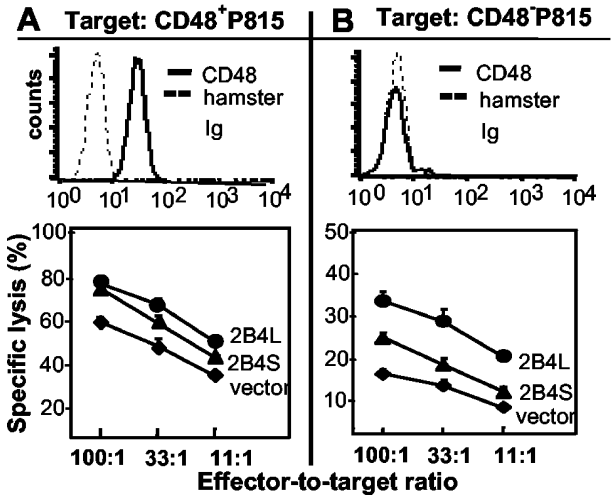

FIGURE 2. Expression of 2B4L or 2B4S augments the cytotoxicity against CD48$^+$ targets as well as CD48$^-$ targets. P3 2C/Rag2$^{-/-}$ T cells transduced with 2B4L, 2B4S, or vector were used as effectors in a 4-h ^{51}Cr-release assay against CD48$^+$P815 (*A*) or CD48$^-$P815 variant (*B*). CD48 expression on these target cells was analyzed by FACS before the assay, and shown on top. Dotted line represents isotype control staining and the solid line represents CD48 staining.

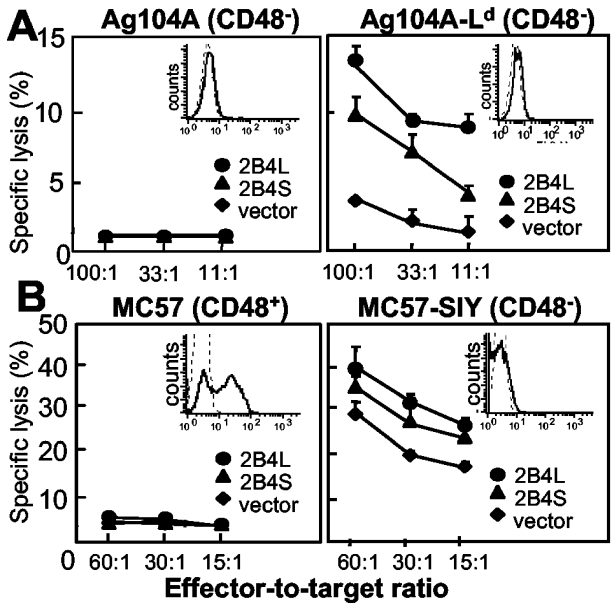

FIGURE 3. Effect of 2B4 overexpression on the cytotoxicity against CD48-negative fibrosarcoma lines, Ag104, and MC57. P3 2C/Rag$^{-/-}$ T cells transduced with 2B4S, 2B4L, or vector were used as effectors in a 5-h ^{51}Cr-release assay. Target cells used are (*A*) naturally occurring mouse fibrosarcoma (K^k), Ag104 (H-2^k), or Ag104-transfected with L^d and (*B*) naturally occurring mouse fibrosarcoma, MC57 (H2^b), or MC57 stably transfected with the 2C antigenic peptide, SIYRYYGL. *Inset*, CD48 expression analyzed by FACS (dotted line, isotype control; solid line, anti-CD48).

cells were two times more potent than vector-transduced cells (compare E:T ratio for 50% lysis). Thus, unlike previous data with NK cells, our results show that both isoforms of 2B4 function as stimulatory molecules.

To determine whether the increased lytic ability of 2B4$^+$ T cells resulted from the ligation of 2B4 with CD48 on target cells, we examined the CTL activity of 2B4-transduced cells against a CD48-negative P815 target (Fig. 2*B*). We expected no augmentation of lytic activity against CD48-negative P815 cells in 2B4-transduced cells but, to our surprise, 2B4 caused strong augmentation of CTL activity against these targets even though the baseline killing with vector-transduced T cells was lower (Fig. 2*B*). Indeed, the relative increase of cytotoxicity was much higher against CD48$^-$P815 targets (Fig. 2*B*) than CD48$^+$P815 targets (Fig. 2*A*). These data suggested that 2B4 engagement with target cells was not necessary for its function.

2B4 costimulates T cell cytotoxicity against CD48-negative tumor targets

To extend these studies to other CD48-negative targets, we examined the lysis of a mouse fibrosarcoma line, Ag104 (H-2^K) and its L^d transfectant. In the absence of the 2C TCR ligand L^d, primed 2C T cells did not lyse Ag104 targets (Fig. 3*A*, *left panel*). Transfection of L^d into Ag104 cells made them susceptible to lysis by 2C T cells, which was further potentiated by overexpression of 2B4L or 2B4S (Fig. 3*A*, *right panel*). Ag104 targets are relatively resistant to CTL lysis in a 4-h ^{51}Cr-release assay; nevertheless, overexpression of 2B4L or 2B4S enhanced the lysis more than 5-fold, suggesting that 2B4 could augment the lysis of resistant targets.

Because 2B4 may mediate non-MHC-restricted lysis through CD48, we examined whether cells expressing CD48 but not a cognate Ag for 2C T cells were susceptible to lysis. For this, we used a syngeneic MC57 tumor line in which ~50% of cells expressed CD48 on their surface (Fig. 2*B*, *left panel*). Neither vector-transduced nor 2B4- transduced T cells lysed MC57 cells, indicating that the target cell lysis was entirely MHC-restricted and that 2B4 only functioned in augmenting this lysis. To further demonstrate this, we tested whether transduced T cells lysed a CD48$^-$MC57 clone that expressed the K^b-restricted ligand for 2C TCR, SIYRYYGL (MC57-SIY) (Fig. 2*B*, *right panel*; Ref. 11). Because this particular line did not express CD48, it could not be lysed through 2B4/CD48 interaction between effector and target cells. As with the CD48$^-$P815 target, MC57-SIY cells were lysed to a greater extent when 2C T cells expressed either isoform of 2B4. Collectively, these data demonstrate that the ligation of 2B4 with CD48 on target cells does not induce non-MHC-restricted lysis by T cells and that 2B4 can only augment TCR-mediated cytotoxicity. Thus, 2B4 acts as a costimulatory receptor in cytotoxic T cells.

Blocking 2B4/CD48 interaction abrogated 2B4-mediated lytic activity

Because the ligation of 2B4 on T cells by CD48 on target cells seems not necessary for the 2B4 function, we considered the possibility that the 2B4-mediated augmentation of lysis was due to 2B4 engagement by CD48 on neighboring T cells. To test this possibility, we examined whether blocking 2B4/CD48 interaction between T cells could bring down the 2B4-enhanced lysis to the basal level (Fig. 4). Consistent with this hypothesis, incubation of T cells with anti-CD48 mAbs during transduction phase significantly reduced 2B4-augmented cytolysis (Fig.

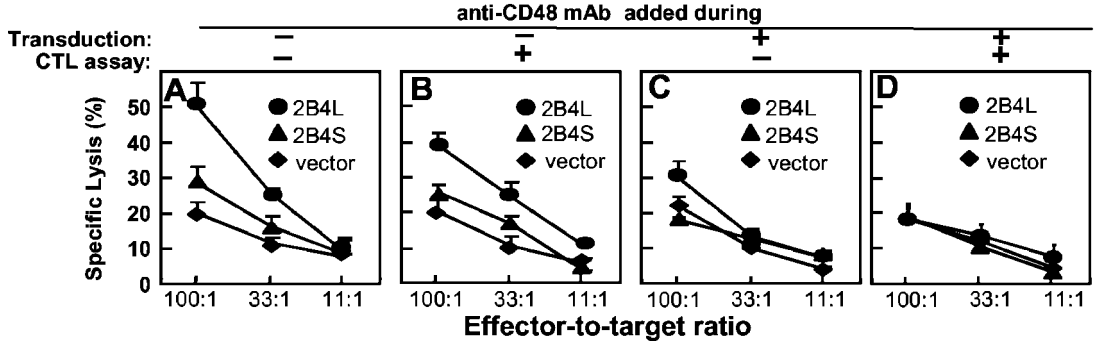

FIGURE 4. Blocking 2B4/CD48 interaction with anti-CD48 Abs reduces 2B4-mediated cytotoxicity. P3 2C/Rag$^{-/-}$ T cells were transduced in the absence (*A* and *B*) or presence (*C* and *D*) of 5 μg/ml anti-CD48 Abs. After transduction, cells were used as effectors in a ^{51}Cr-release assay against SIY-MC57 targets (5 h). *B* and *D*, Anti-CD48 Abs (5 μg/ml) were included in the 5-h ^{51}Cr-release assay.

4*C*) whereas addition of anti-CD48 mAbs during the ^{51}Cr-release assay had little effect on cytolysis (Fig. 4*B*). Continuous blocking of 2B4/CD48 interaction by addition of anti-CD48 mAb during the transduction phase and a 4-h ^{51}Cr-release assay almost completely inhibited 2B4-enhanced cytotoxicity (Fig. 4*D*). This inhibitory effect was not due to altered 2B4 expression on T cells as addition of anti-CD48 mAb in the culture during the transduction period did not change the level of 2B4 expression (data not shown). Rather it suggests that 2B4/CD48 interaction among T cells during the induction phase of 2B4 expression can give rise to increased T cell cytotoxicity against CD48$^-$ target cells. Taken together, our data demonstrate that the 2B4-stimulated CTL response was mediated through interaction of 2B4 with CD48 expressed on the 2CRag2$^{-/-}$ T cells themselves and, thus, this effect could be blocked by preventing 2B4/CD48 interactions before target cell exposure.

Discussion

2B4 is expressed on all NK cells and cross-linking of 2B4 enhances non-MHC-restricted cytotoxicity. In this study, we demonstrate a novel costimulatory function of 2B4 in enhancing MHC-restricted target cell lysis by CTL. This costimulatory function of 2B4 was mediated through its interaction with CD48 which is known to be expressed on all T cells. We propose that during T cell priming 2B4/CD48 interactions among neighboring T cells activate their lytic function. As in other models of costimulation, our data show that 2B4/CD48 interactions between T cells (signal 2) occur concurrently with the engagement of 2C TCR (signal 1) by H-2L^d expressing P815.B71 cells during priming of the 2C T cells.

A recent study using influenza virus-specific TCR transgenic mice showed that 2B4/CD48 interactions caused increased proliferation of CD8$^+$ T cells (5). In this study, it was found that 2B4 functioned as a stimulatory ligand for CD48 on the neighboring T cells. Thus, it is possible that the increased lysis by 2B4$^+$ T cells might also have been due to the signaling through CD48 on neighboring T cells. Alternatively, upon ligation with CD48, 2B4 itself might have generated activating signals to augment MHC-restricted lysis. The fact that 2B4L, whose extracellular ligand binding domain is identical to that of 2B4S, exhibited greater augmentation in cytolysis than 2B4S (Figs. 2–4) does not favor the possibility that CD48 functioned as a costimulatory receptor. Moreover, in preliminary experiments we have found that expression of 2B4 on target cells failed to augment their lysis by CD48$^+$2B4$^-$ T cells, consistent with the idea that 2B4, not CD48, is an activating receptor for cytolysis. In further support of this notion, anti-2B4 mAb cross-linking in the presence of a suboptimal dose of anti-CD3 mAb in 2B4-transduced 2C T cells caused augmentation of proliferation (data not shown), suggesting that 2B4 can function as a costimulating receptor in T cell proliferation. However, it is also possible that the difference seen with 2B4L vs 2B4S might have been simply due to the level of surface expression, in that higher expression caused stronger ligation of CD48 and hence higher cytotoxicity. Hence, additional experiments are required to define the molecular mechanism by which 2B4 stimulated cytolysis in T cells. In other studies,[4] we have found that 2B4$^+$ T cells isolated from normal spleens proliferate faster in response to cytokines, and upon ligation of 2B4 produce a higher level of IFN-γ. Thus, 2B4$^+$ T cells isolated from spleens are also functionally different from 2B4$^-$ T cells.

Our previous studies with mouse NK cells did not address whether 2B4 acts as a triggering receptor or a coreceptor for other activating receptors (2). Recent findings in human NK cells demonstrated a costimulatory role of 2B4 in triggering natural cytotoxicity receptor-mediated cytotoxicity (15). Similarly, Nakajima et al. (3) have reported the variable effect of 2B4 in stimulating cytolysis among different human NK cell clones, suggesting the relative contribution of other receptors to 2B4-mediated killing. Likewise, 2B4 expressed on human CD8$^+$ T cells also failed to trigger cytolytic activity in a redirected killing assay (3), confirming that 2B4 does not directly stimulate non-MHC-restricted cytolysis in T cells. Our data on murine CD8$^+$ T cells also favor a coreceptor role of 2B4. Therefore, it is likely that the primary function of 2B4 in both NK and T cells may be to assist other activating receptors in target cell lysis.

In our previous studies, transfection of 2B4S or 2B4L into the RNK-16 rat leukemic cell line revealed an opposing function of 2B4S and 2B4L in target cell lysis (4). In contrast, present data in CD8$^+$ T cells show that both forms of 2B4 had a costimulatory role. In both NK and T cells, 2B4S appears to be stimulatory whereas 2B4L in RNK-16 cells was inhibitory but in T cells it was stimulatory. It is not clear why the same molecule functions differently in NK and T cells. Recent data in human NK cells suggests that 2B4L (the only form expressed in human) can act as an activating or inhibitory receptor depending on the availability of an adaptor molecule, SLAM-associated protein (SAP) (16). Thus, in developing human NK cells, the intracellular levels of functional SAP are low, which may result in an inhibitory role of 2B4. It is thought that SAP functions as an inhibitor of a tyrosine phosphatase, Src homology protein-2, by preventing binding of Src homology protein-2 to SLAM (17) and 2B4 (18). However, more recent studies have revealed that SAP can also actively facilitate recruitment of a tyrosine kinase, Fyn T, to the SLAM receptor, and promote phosphorylation of downstream effector molecules (19). Thus, the relative contribution of these signaling molecules, rather than the 2B4 isoforms, may be the critical determinant of 2B4 function. Detailed analysis of the intracellular signaling pathway of 2B4L in NK and T cells will help to elucidate the distinct function of 2B4 in NK and T cells.

To our knowledge, our data provide the first piece of evidence for the presence of costimulation among T cell themselves in MHC-restricted lysis. Because 2B4-dependent costimulation does not depend on the expression of CD48 on target cells, the 2B4/CD48 pair provides a more versatile costimulation system, which may exert its function when 2B4 is up-regulated on activated/memory CD8$^+$ T cells. Furthermore, 2B4/CD48 may represent a unique pair of receptors which can signal in both directions and induce cytotoxicity and proliferation. Therefore, engineering 2B4 expression on T cells may be a novel strategy to facilitate tumor immunotherapy for patients whose CD8$^+$ T cells are nonresponsive due to poor costimulation or poor Ag expression.

[4] J. Klem, K.-M. Lee, L. Greenlee, J. Mooney, S. Bhawan, V. Kumar, and J. D. Schatzle. 2B4 (CD244) defines a unique CD8$^+$ activated/memory T cell subset with enhanced IFN γ production and susceptibility to apoptosis. *Submitted for publication.*

Note added in proof. Since the submission of this publication, it has come to our attention that a recent study by Stefanova et al. (20) has published complimentary findings.

Acknowledgments

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References

1. Mathew, P. A., B. A. Garni-Wagner, K. Land, A. Takashima, E. Stoneman, M. Bennett, and V. Kumar. 1993. Cloning and characterization of the 2B4 gene encoding a molecule associated with non-MHC-restricted killing mediated by activated natural killer cells and T cells. *J. Immunol. 151:5328.*
2. Garni-Wagner, B. A., A. Purohit, P. A. Mathew, M. Bennett, and V. Kumar. 1993. A novel function-associated molecule related to non-MHC-restricted cytotoxicity mediated by activated natural killer cells and T cells. *J. Immunol. 151:60.*
3. Nakajima, H., M. Cella, H. Langen, A. Friedlein, and M. Colonna. 1999. Activating interactions in human NK cell recognition: the role of 2B4-CD48. *Eur. J. Immunol. 29:1676.*
4. Schatzle, J. D., S. Sheu, S. E. Stepp, P. A. Mathew, M. Bennett, and V. Kumar. 1999. Characterization of inhibitory and stimulatory forms of the murine natural killer cell receptor 2B4. *Proc. Natl. Acad. Sci. USA 96:3870.*
5. Kambayashi, T., E. Assarsson, B. J. Chambers, and H. G. Ljunggren. 2001. Cutting edge: regulation of $CD8^+$ T cell proliferation by 2B4/CD48 interactions. *J. Immunol. 167:6706.*
6. Sha, W. C., C. A. Nelson, R. D. Newberry, D. M. Kranz, J. H. Russell, and D. Y. Loh. 1988. Selective expression of an antigen receptor on CD8-bearing T lymphocytes in transgenic mice. *Nature 335:271.*
7. Udaka, K., T. J. Tsomides, and H. N. Eisen. 1992. A naturally occurring peptide recognized by alloreactive $CD8^+$ cytotoxic T lymphocytes in association with a class I MHC protein. *Cell 69:989.*
8. Rivas, F. V., S. O'Herrin, and T. F. Gajewski. 2001. CD28 is not required for c-Jun N-terminal kinase activation in T cells. *J. Immunol. 167:3123.*
9. Chen, L., P. McGowan, S. Ashe, J. Johnston, Y. Li, I. Hellstrom, and K. E. Hellstrom. 1994. Tumor immunogenicity determines the effect of B7 costimulation on T cell-mediated tumor immunity. *J. Exp. Med. 179:523.*
10. Hahn, K., R. DeBiasio, A. Tishon, H. Lewicki, J. E. Gairin, G. LaRocca, D. L. Taylor, and M. Oldstone. 1994. Antigen presentation and cytotoxic T lymphocyte killing studied in individual, living cells. *Virology 201:330.*
11. Spiotto, M. T., P. Yu, D. A. Rowley, M. I. Nishimura, S. C. Meredith, T. F. Gajewski, Y.-X. Fu, and H. Schreiber. Tumor stroma cross-presents highly expressed antigens to overcome ignorance to solid tumors. *Immunity 17:737.*
12. Morita, S., T. Kojima, and T. Kitamura. 2000. Plat-E: an efficient and stable system for transient packaging of retroviruses. *Gene Ther. 7:1063.*
13. Clay, T. M., M. C. Custer, J. Sachs, P. Hwu, S. A. Rosenberg, and M. I. Nishimura. 1999. Efficient transfer of a tumor antigen-reactive TCR to human peripheral blood lymphocytes confers anti-tumor reactivity. *J. Immunol. 163:507.*
14. Lee, K. M., E. Chuang, M. Griffin, R. Khattri, D. K. Hong, W. Zhang, D. Straus, L. E. Samelson, C. B. Thompson, and J. A. Bluestone. 1998. Molecular basis of T cell inactivation by CTLA-4. *Science 282:2263.*
15. Sivori, S., S. Parolini, M. Falco, E. Marcenaro, R. Biassoni, C. Bottino, L. Moretta, and A. Moretta. 2000. 2B4 functions as a co-receptor in human NK cell activation. *Eur. J. Immunol. 30:787.*
16. Sivori, S., M. Falco, E. Marcenaro, S. Parolini, R. Biassoni, C. Bottino, L. Moretta, and A. Moretta. 2002. Early expression of triggering receptors and regulatory role of 2B4 in human natural killer cell precursors undergoing in vitro. *Proc. Natl. Acad. Sci. USA 99:4526.*
17. Sayos, J., C. Wu, M. Morra, N. Wang, X. Zhang, D. Allen, S. van Schaik, L. Notarangelo, R. Geha, M. G. Roncarolo, et al. 1998. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature 395:462.*
18. Tangye, S. G., S. Lazetic, E. Woollatt, G. R. Sutherland, L. L. Lanier, and J. H. Phillips. 1999. Cutting edge: human 2B4, an activating NK cell receptor, recruits the protein tyrosine phosphatase SHP-2 and the adaptor signaling protein. *J. Immunol. 162:6981.*
19. Latour, S., G. Gish, C. D. Helgason, R. K. Humphries, T. Pawson, and A. Veillette. 2001. Regulation of SLAM-mediated signal transduction by SAP, the X-linked lymphoproliferative gene product. *Nat. Immunol. 2:681.*
20. Stefanova, I., B. Hemmer, M. Vergelli, R. Martin, W. Biddison, and R. Germain. 2003. TCR ligand discrimination is enforced by competing ERK positive and SHP-1 negative feedback pathways. *Nat. Immunol. 248:54.*