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Cutting Edge: NK Cell Inhibitory Receptors Prevent Tyrosine Phosphorylation of the Activation Receptor 2B4 (CD244)[1]

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2B4 is an NK cell activation receptor that can provide a costimulatory signal to other activation receptors and whose mode of signal transduction is still unknown. We show that cross-linking of 2B4 on NK cells results in its rapid tyrosine phosphorylation, implying that this initial step in 2B4 signaling does not require coligation of other receptors. Ligation of 2B4 in the context of an NK cell-target cell interaction leads to 2B4 tyrosine phosphorylation, target cell lysis, and IFN-γ release. Coligation of 2B4 with the inhibitory receptors killer cell Ig-like receptor (KIR)2DL1 or CD94/NKG2 completely blocks NK cell activation. The rapid tyrosine phosphorylation of 2B4 observed upon contact of NK cells with sensitive target cells is abrogated when KIR2DL1 or CD94/NKG2 are engaged by their cognate MHC class I ligand on resistant target cells. These results demonstrate that NK inhibitory receptors can interfere with a step as proximal as phosphorylation of an activation receptor. ***The Journal of Immunology*****, 2000, 165: 3545–3548.**

Natural killer cell function is regulated by a dynamic balance between negative and positive signals. Receptors for MHC class I molecules are involved mainly in negative regulation of NK cell-mediated cytotoxicity (1, 2). The inhibitory nature of those receptors is determined by an immunoreceptor tyrosine-based inhibition motif (ITIM)[3] that recruits the tyrosine phosphatase SHP-1 upon phosphorylation (3). The activity of SHP-1 is essential to terminate NK cell cytotoxicity. However, little is known about the mechanism of inhibition of NK cell activation during natural killing. Ab-mediated co-cross-linking of inhibitory receptors with CD16 inhibits the phosphorylation of Fc receptor-associated ζ signaling chain and of more downstream signaling molecules like ZAP-70, SLP-76, and phospholipase C (PLC)γ (4, 5). However, engagement of inhibitory receptors on NK cells or $\gamma\delta$ T cells during target cell contact affects phosphorylation of LAT, Lck, and ZAP-70, but not that of the ζ-chain (6, 7). Therefore, it is not known whether inhibition of target cell lysis through MHC class I-specific receptors acts at the level of activation receptors or by targeting downstream effector molecules.

The activating NK cell receptors NKp46, NKp44, NKp30, and 2B4 have been identified recently (8, 9). Whereas NKp46, NKp44, and NKp30 pair with immunoreceptor tyrosine-based activation motif-containing partner chains, 2B4 activates NK cells by an unknown mechanism. 2B4 can function as a costimulatory receptor for other immunoreceptor tyrosine-based activation motif-based activation receptors (10). 2B4 induces secretion of IFN-γ and IL-2, granule exocytosis, and cell-mediated cytotoxicity (11–13). The ligand for mouse and human 2B4 is CD48, a GPI-linked molecule expressed mostly on hematopoietic cells (14, 15).

In this paper, we investigated how engagement of NK inhibitory receptors during NK:target cell contact may affect the 2B4 receptor.

Materials and Methods

Cells and Abs

Human NK populations were isolated from human PBL using the magnetic-activated cell sorter NK cell isolation kit (Miltenyi Biotec, Auburn, CA). Populations were between 90%–99% $CD3^-$, $CD56^+$, and $2B4^+$. Cells used in this study were the human NK cell lines YTS-2DL1 (Ref. 16; gift from G. Cohen, Charlestown, MA), YTS-2DL1-SHP-1, YTS-2DL1-SHP-$1^{(RM)}$ (17), NKL (Ref. 18; gift from M. J. Robertson, Indianapolis, IN), 721.221, 221-Cw3, 221-Cw4, 221-Cw7 (a gift from J. Gumperz, Boston, MA, and P. Parham, Stanford, CA), and P815 (American Type Culture Collection, Manassas, VA).

The following Abs were used: anti-2B4 (C1.7, IgG1; Coulter Pharmaceutical, Miami, FL), rabbit anti-2B4 (generated against the peptide RLSR KELENFDVYS; Research Genetics, Huntsville, AL), MOPC-21 (Sigma, St. Louis, MO) as a mouse IgG1 control, anti-CD94 (HP-3D9; Ancell, Bayport, MN), anti-CD16 (3G8; Medarex, Annandale, NJ), anti-CD48 (10H3; a gift from F. Mami-Chouaib; Ref. 19), anti-killer cell Ig-like receptor (KIR)2DL1 (EB6, IgG1 (Coulter Pharmaceutical) and HP-3E4, IgM (a gift from M. López-Botet); Ref. 20), anti-SHP-1 (Transduction Laboratories, Lexington, KY), anti-PLCγ1 (B-6–4, mouse IgG1), anti-vav, and the biotin-conjugated anti-phosphotyrosine specific Ab 4G10 (IgG2b; all Upstate Biotechnology, Lake Placid, NY).

^{51}Cr release assay and ELISA

A standard 3-h ^{51}Cr release assay was performed as previously described (21). For IFN-γ release, 1×10^5 NK cells were incubated with 1 μg of the

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[3] Abbreviations used in this paper: ITIM, immunoreceptor tyrosine-based inhibition motif; KIR, killer cell Ig-like receptor; PLC, phospholipase C.

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indicated Abs and 1×10^5 target cells (P815 or 721.221) in 250 μl medium for 20 h at 37°C. Supernatants were harvested and analyzed in duplicates in an IFN-γ-specific ELISA (Genzyme, Cambridge, MA) according to the manufacturer's instructions.

Receptor cross-linking, cell mixing, immunoprecipitation, and Western blotting

For Ab-mediated cross-linking of 2B4, 5×10^6 human NK cells or YTS-2DL1 were incubated with 1 μg Ab in 100 μl medium for 10 min on ice. After the addition of 2 μg goat anti-mouse Abs, cells were transferred to 37°C for the indicated times and subsequently lysed in ice cold lysis buffer (0.5% Triton X-100, 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 10% Glycerin, 2 mM EDTA, 1 mM PMSF, 10 mM NaF, and 1 mM $NaVO_4$). Abs were then immunoprecipitated using Protein G agarose (Life Technologies, Rockville, MD).

For cell mixing, NK cells and target cells were mixed in 200 μl medium at an E:T ratio of 1 (YTS-2DL1 and NKL) or 2 (human NK cells) and pelleted by centrifugation. Cells were incubated on ice for 10 min, transferred to 37°C for the indicated times, and lysed as described above. Lysates were first incubated with 2 μg control IgG1 coupled to Protein G agarose followed by 2 μg anti-2B4 or 2 μg anti-SHP-1 (both coupled to Protein G agarose). For Western blotting, samples were separated on a 10–20% SDS gel (NOVEX, San Diego, CA), transferred to a polyvinylidene difluoride membrane (Immobilon P; Millipore, Bedford, MA), and developed using biotinylated 4G10, peroxidase-coupled streptavidin (Amersham, Arlington Heights, IL), and Super Signal West Dura Extended Duration substrate (Pierce, Rockford, IL).

Results and Discussion

2B4 can trigger NK cell activation

The cytoplasmic tail of 2B4 contains 4 tyrosine-based sequence motifs (TxYxxI/V) that are also present in CD150 (signaling lymphocytic activation molecule), CD84, and Ly9. Ab-mediated cross-linking of 2B4 resulted in its rapid tyrosine phosphorylation, which peaked at about 3 min after receptor engagement (Fig. 1*A*). Ab-mediated cross-linking of 2B4 also led to the phosphorylation of several cytoplasmic proteins (Fig. 1*B*), including PLCγ1 and vav (Fig. 1*C*), both of which have been implicated in the signal transduction leading to natural cytotoxicity (6, 22). In a redirected lysis assay with the FcR-positive mouse cell line P815, cross-linking of 2B4 induced cellular cytotoxicity by purified human NK cells (Fig. 1*D* and Ref. 11). Lysis of P815 was not observed using an isotype-matched control Ab and was similar to lysis induced by Ab binding to an activating form of CD94/NKG2 but less than that obtained with an anti-CD16 mAb (Fig. 1*D*). In this type of redirected assay, 2B4-mediated NK cell activation depends on coengagement of the NKp46 receptor that can recognize an unknown ligand expressed on the mouse cell line P815 (10). It is not known how the signals of NKp46 and 2B4 are integrated during NK cell activation. However, 2B4 phosphorylation is not dependent on NKp46 or other activating receptors, as it could be induced directly by Ab-mediated cross-linking of 2B4 (Fig. 1*A*). The SH2 domain-containing molecule SH2D1A (SLAM-associated protein, DSHP) binds to phosphorylated 2B4 and competes for association of the tyrosine phosphatase SHP-2 (23). However, it is unknown how SH2D1A or SHP-2 contribute to 2B4 signaling and the activation signal delivered by 2B4 may also depend on association of other molecules. Tyrosine phosphorylation is required for 2B4 signaling as the Src-family tyrosine kinase inhibitors herbimycin A and PP1, and the syk inhibitor piceatannol interfered with 2B4-mediated killing by purified human NK cells in a redirected lysis experiment (data not shown).

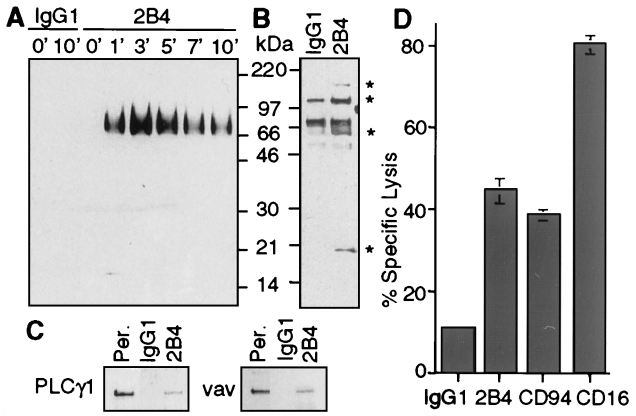

FIGURE 1. Activation of NK cells by 2B4. *A*, 2B4 immunoprecipitation following Ab cross-linking using 5×10^6 purified human NK cells with 1 μg control IgG1 (MOPC 21) or anti-2B4 (C1.7) for the indicated time. *B*, Ab cross-linking of 2B4 in human NK populations for 10 min. Lysate equivalent to 5×10^5 cells was analyzed. *C*, Purified human NK cells were treated with pervanadate (Per.) or stimulated with control IgG1 or anti-2B4 for 10 min as described in *A*. Vav or PLCγ1 was immunoprecipitated. *A–C*, All samples were analyzed by anti-phosphotyrosine Western blotting. *D*, Redirected lysis assay of P815 cells using purified human NK cells expressing an activating CD94/NKG2 receptor in the presence of 0.5 μg/ml control IgG1 (MOPC 21), anti-2B4 (C1.7), anti-CD94 (HP3D9) or anti-CD16 (3G8). Lysis is shown for an E:T ratio of 2.

Inhibition of 2B4-mediated NK cell activation by KIR and CD94/NKG2A

The NK cell line YTS stably transfected with the inhibitory KIR2DL1 (YTS-2DL1; Ref. 16) and the cell line NKL (18) that expresses an inhibitory form of CD94/NKG2 (24) were used to test whether 2B4-mediated cytotoxicity can be blocked by inhibitory receptors. In a redirected lysis assay, stimulation of 2B4 on YTS-2DL1 and NKL led to cellular cytotoxicity that was completely inhibited by coligation with KIR2DL1 or CD94/NKG2A, respectively (Fig. 2, *A* and *B*; Ref. 25). These results show that inhibition is dominant over 2B4-mediated NK cell activation. A similar inhibition could be observed using purified human NK cells expressing an inhibitory form of CD94/NKG2 (data not shown).

2B4 was also able to stimulate IFN-γ secretion by the NK cell line NKL (Fig. 2*C*) and by human NK cells (Ref. 11 and data not shown). As reported previously (11), Ab-mediated cross-linking of 2B4 alone resulted in only minimal IFN-γ secretion. Much greater IFN-γ secretion occurred when the anti-2B4 Ab was presented by

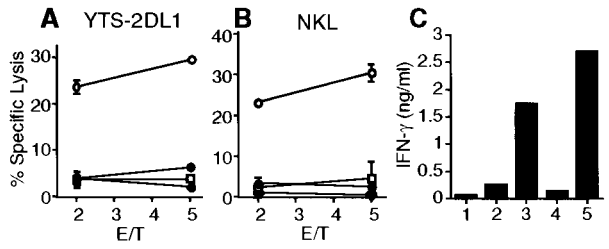

FIGURE 2. Inhibition of 2B4-mediated killing and IFN-γ production by KIR and CD94. *A*, Redirected lysis of P815 cells using the NK cell line YTS-2DL1 in the presence of control IgG1 (□), anti-2B4 plus control IgG1 (○), anti-2DL1 plus control IgG1 (♦), or anti-2DL1 plus anti-2B4 (●). *B*, Redirected lysis of P815 cells using NKL cells in the presence of control IgG1 (□), anti-2B4 plus control IgG1 (○), anti-CD94 plus control IgG1 (♦), or anti-CD94 plus anti-2B4 (●) (all Abs at 0.5 μg/ml each). All experiments were performed at least three times in triplicates; mean and SD are shown. *C*, NKL cells were incubated with medium alone (*1*), 1 μg anti-2B4 plus goat anti-mouse (*2*), P815 cells with 1 μg anti-2B4 plus 1 μg control IgG1 (*3*), P815 cells with 1 μg anti-2B4 plus 1 μg anti-CD94 (*4*), or 721.221 cells (*5*). Supernatants were harvested and analyzed by ELISA for IFN-γ.

the FcγR$^+$ cell line P815. This indicates that NK cells need additional signals, such as engagement of NKp46 (10), along with 2B4 signals to be fully activated. As already observed for killing, coligation of 2B4 with CD94 on NKL cells resulted in the inhibition of IFN-γ secretion (Fig. 2*C*).

Inhibition of target cell-induced 2B4 phosphorylation by KIR and CD94/NKG2

The requirement for tyrosine kinases in 2B4-mediated activation signals and the tyrosine phosphorylation of 2B4 upon receptor cross-linking suggested that receptor phosphorylation may be an important step in 2B4 signaling. Therefore, phosphorylation of 2B4 in the physiological setting of target cell contact was evaluated. The MHC class I-negative target cell 721.221 expresses CD48 (data not shown). Mixing of YTS-2DL1 cells with 721.221 led to 2B4 phosphorylation that was detectable as early as 30 s after target cell contact (Fig. 3*A*). These results suggest that 2B4 is engaged during contact with CD48-positive target cells and that it may play a role in NK cell activation. Treatment of target cells with the blocking anti-CD48 mAb 10H3 (19) led to about 50% reduction of cytotoxicity by the NK cell line YTS-2DL1 against the target cell 221-Cw3 (data not shown). This confirms that 2B4 may be one among other receptors involved in natural killing by NK cells.

As shown in Fig. 2, inhibitory receptors can interfere with 2B4-mediated NK cell activation. However, little is known about the step at which inhibitory receptors exert their function. The influence of inhibitory receptors on 2B4 signaling was investigated. KIR2DL1 recognizes HLA-Cw4 but not HLA-Cw3 (21, 26). Therefore, the KIR2DL1-transfected NK cell line YTS-2DL1 lysed cells expressing HLA-Cw3 (221-Cw3) but not 721.221 cells expressing HLA-Cw4 (221-Cw4; Fig. 3*B*). This inhibition of YTS-2DL1 was reverted by a blocking Ab against KIR2DL1 (Fig. 3*B*). 221-Cw3 and 221-Cw4 cells express the same level of CD48 (data not shown). As shown in Fig. 3*C*, incubation of YTS-2DL1 with 221-Cw3 led to strong and rapid 2B4 phosphorylation. This 2B4 phosphorylation could not be observed when YTS-2DL1 was mixed with the resistant target 221-Cw4, indicating that the inhibitory signal by KIR2DL1 interferes with 2B4-mediated NK cell activation directly at the level, or even upstream of receptor phosphorylation. 2B4 phosphorylation was observed upon Ab-mediated cross-linking of the receptor alone without a need to cross-link other activating receptors (Fig. 1*A*). However, 2B4 phosphorylation during target cell contact may still be influenced by other activating receptors, such as NKp46. Therefore, inhibition of 2B4 phosphorylation during contact with resistant target cells could be mediated by a block at the level of other activating receptors. To test whether the direct phosphorylation of 2B4 could be prevented by an inhibitory receptor 2B4 and KIR2DL1 were cocross-linked with Abs. A similar inhibition of 2B4 phosphorylation by KIR2DL1 was observed (Fig. 3*E*), demonstrating that KIR can directly inhibit 2B4 phosphorylation.

Inhibitory KIR recruit the phosphatase SHP-1 upon phosphorylation of the cytoplasmic ITIMs (27). SHP-1 can be phosphorylated by Src-family tyrosine kinases located at the plasma membrane (28). Phosphorylation of SHP-1 was detected in YTS-2DL1 mixed with 221-Cw4 but not with 221-Cw3 (Fig. 3*D*). Tyrosine phosphorylation of KIR2DL1 and association of SHP-1 after cell mixing were not detected consistently (data not shown). To test more directly whether SHP-1 may be involved in blocking 2B4 phosphorylation we used YTS cells expressing a chimeric molecule consisting of KIR2DL1 in which the ITIMs in the cytoplasmic tail have been replaced by full-length SHP-1 (YTS-2DL1-SHP-1) or an inactive mutant of SHP-1 (YTS-2DL1-SHP-1$^{(RM)}$) (17). In cell mixing experiments, engagement of the chimeric KIR2DL1-SHP-1 receptor by HLA-Cw4-expressing target cells blocked 2B4

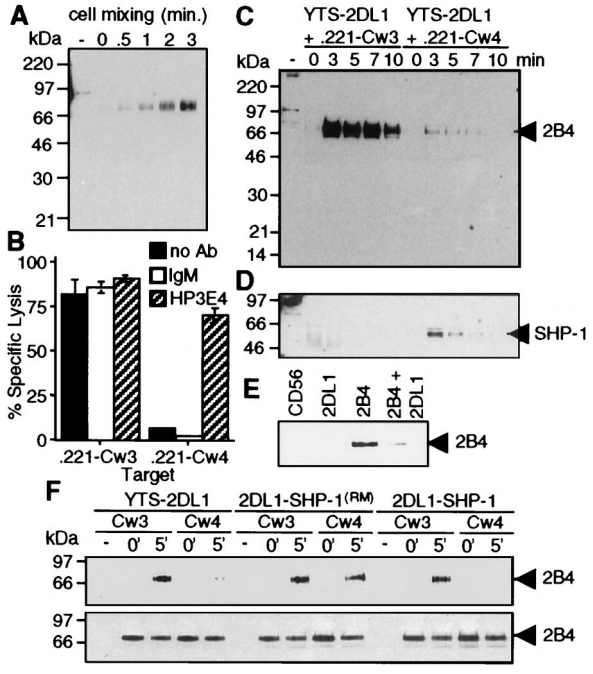

FIGURE 3. Inhibition of 2B4 phosphorylation by KIR. *A*, The NK cell line YTS-2DL1 was mixed with the target 721.221 for the indicated times. Cell lysates were immunoprecipitated with control IgG1 (-, 3-min sample shown) followed by anti-2B4. *B*, A 3-h ^{51}Cr release assay using YTS-2DL1 cells and the indicated targets in the presence of no Ab, a control IgM ascites (1:1000) or anti-KIR (HP-3E4) ascites (1:1000) as indicated. Lysis is shown for an E:T ratio of 5. *C* and *D*, YTS-2DL1 cells were mixed with the target cell 221-Cw3 or 221-Cw4 at an E:T ratio of 1 for the indicated time. Cells were lysed and immunoprecipitated with a control IgG1 (-, 3-min sample shown) followed by anti-2B4 (*C*) or anti-SHP-1 (*D*). *E*, Immunoprecipitation of 2B4 following Ab-mediated cross-linking of the indicated receptors on YTS-2DL1 cells. The Ab concentration between samples was kept constant by the addition of anti-CD56 Ab. *A* and *C–E*, All samples were analyzed by anti-phosphotyrosine Western blotting. *F*, The NK cell lines YTS-2DL1, YTS-2DL1-SHP-1, and YTS-2DL1-SHP-1$^{(RM)}$ were mixed with target cells as described in *C*. Cells were lysed and immunoprecipitated with a control IgG1 (-, 5-min sample shown) followed by anti-2B4. Samples were analyzed by anti-phosphotyrosine Western blotting (upper panel) and reprobed with an anti-2B4 Ab (lower panel).

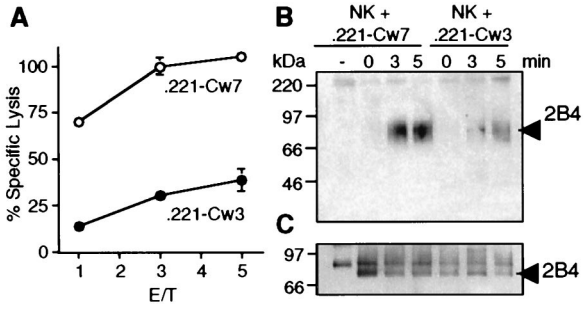

FIGURE 4. Inhibition of 2B4 phosphorylation by CD94. *A*, A 4-h ^{51}Cr release assay using purified human NK cells and the indicated target cells. *B*, Purified human NK cells (1×10^7 per sample) were incubated with 221-Cw3 or 221-Cw7 cells at an E:T ratio of 2 for the indicated time. Cells were lysed and immunoprecipitated with a control IgG1 (-, 3-min sample shown) followed by anti-2B4. Immunoprecipitates were analyzed by anti-phosphotyrosine Western blotting. *C*, To confirm equal loading, the Western blot shown in *B* was reprobed with an anti-2B4 Ab. The migration position of 2B4 is indicated.

phosphorylation (Fig. 3*F*). This effect was dependent on the activity of SHP-1 as 2B4 phosphorylation was not blocked in YTS-2DL1-SHP-1$^{(RM)}$ cells (Fig. 3*F*). Therefore, it is possible that SHP-1 is responsible for the direct dephosphorylation of 2B4. Consistent with this we could show dephosphorylation of in vivo phosphorylated 2B4 by recombinant SHP-1 in vitro (data not shown). Inhibition at the level of receptor phosphorylation could explain the KIR-mediated block in phosphorylation of downstream signaling molecules such as LAT, SLP-76, and PLCγ, as observed in earlier studies (4–6).

To validate our findings, the effect of inhibitory receptors on 2B4 phosphorylation was investigated in purified human NK cells. Human NK populations express almost homogenously the C-type lectin-like receptor heterodimer CD94/NKG2 (29) specific for HLA-E (1). HLA-Cw3 and -Cw7 are both recognized by the same KIR receptors. However, whereas CD94/NKG2 binds to HLA-E presenting the leader peptide of HLA-Cw3, it does not bind to HLA-E presenting the leader peptide of HLA-Cw7 (30). Some human NK populations lysed 221-Cw7 but not 221-Cw3 cells, indicating a predominance of CD94/NKG2A on those cells (Fig. 4*A*). 2B4 became phosphorylated in purified human NK cells mixed with the sensitive target cell 221-Cw7 but not with the resistant target 221-Cw3 (Fig. 4*B*). Therefore, as observed with KIR2DL1, the inhibitory CD94 receptor is able to block 2B4-mediated NK cell activation directly at the level or upstream of 2B4 phosphorylation.

Conclusion

The block of 2B4 phosphorylation described in this paper is, to our best knowledge, the first observation of a KIR- or CD94/NKG2-mediated inhibition of the phosphorylation of an activating receptor upon target cell contact. This finding illustrates how inhibitory receptors can interfere early and effectively with NK cell activation. An effect of KIR on 2B4 phosphorylation was detected as early as 1 min after target cell contact (data not shown). In addition, by blocking positive signals right at the region of contact between NK and target cells, inhibitory receptors can mediate a locally defined inhibition of NK cell activation rather than a global one. Consistent with this, a recent report showed that a single NK cell can be in contact with a sensitive and a protected target cell a the same time and kill the sensitive target while sparing the protected one (31). This enables NK cells to detect and kill single target cells in an environment of cells that are protected from the NK cell attack.

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References

1. Lanier, L. L. 1998. NK cell receptors. *Annu. Rev. Immunol. 16:359.*
2. Long, E. O. 1999. Regulation of immune responses through inhibitory receptors. *Annu. Rev. Immunol. 17:875.*
3. Burshtyn, D. N., and E. O. Long. 1997. Regulation through inhibitory receptors: lessons from natural killer cells. *Trends Cell Biol. 7:473.*
4. Binstadt, B. A., K. M. Brumbaugh, C. J. Dick, A. M. Scharenberg, B. L. Williams, M. Colonna, L. L. Lanier, J. P. Kinet, R. T. Abraham, and P. J. Leibson. 1996. Sequential involvement of Lck and SHP-1 with MHC-recognizing receptors on NK cells inhibits FcR-initiated tyrosine kinase activation. *Immunity 5:629.*
5. Binstadt, B. A., D. D. Billadeau, D. Jevremovic, B. L. Williams, N. Fang, T. L. Yi, G. A. Koretzky, R. T. Abraham, and P. J. Leibson. 1998. SLP-76 is a direct substrate of SHP-1 recruited to killer cell inhibitory receptors. *J. Biol. Chem. 273:27518.*
6. Valiante, N. M., J. H. Phillips, L. L. Lanier, and P. Parham. 1996. Killer cell inhibitory receptor recognition of human leukocyte antigen (HLA) class I blocks formation of a pp36/PLC-γ signaling complex in human natural killer (NK) cells. *J. Exp. Med. 184:2243.*
7. Carena, I., A. Shamshiev, A. Donda, M. Colonna, and G. D. Libero. 1997. Major histocompatibility complex class I molecules modulate activation threshold and early signaling of T cell antigen receptor-γ/δ stimulated by nonpeptidic ligands. *J. Exp. Med. 186:1769.*
8. Lanier, L. L. 2000. Turning on natural killer cells. *J. Exp. Med. 191:1259.*
9. Moretta, A., R. Biassoni, C. Bottino, M. C. Mingari, and L. Moretta. 2000. Natural cytotoxicity receptors that trigger human NK-cell-mediated cytolysis. *Immunol. Today 21:228.*
10. 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.*
11. Valiante, N. M., and G. Trinchieri. 1993. Identification of a novel signal transduction surface molecule on human cytotoxic lymphocytes. *J. Exp. Med. 178:1397.*
12. 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.*
13. 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.*
14. Latchman, Y., P. F. McKay, and H. Reiser. 1998. Identification of the 2B4 molecule as a counter-receptor for CD48. *J. Immunol. 161:5809.*
15. Brown, M. H., K. Boles, P. A. Van der Merwe, V. Kumar, P. A. Mathew, and A. N. Barclay. 1998. 2B4, the natural killer and T cell immunoglobulin superfamily surface protein, is a ligand for CD48. *J. Exp. Med. 188:2083.*
16. Cohen, G. B., R. T. Gandhi, D. M. Davis, O. Mandelboim, B. K. Chen, J. L. Strominger, and D. Baltimore. 1999. The selective down-regulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. *Immunity 10:661.*
17. Burshtyn, D. N., J. Shin, C. C. Stebbins, and E. O. Long. 2000. Adhesion to target cells is disrupted by the killer cell inhibitory receptor. *Curr. Biol. 10:777.*
18. Robertson, M. J., K. J. Cochran, C. Cameron, J. M. Le, R. Tantravahi, and J. Ritz. 1996. Characterization of a cell line, NKL, derived from an aggressive human natural killer cell leukemia. *Exp. Hematol. 24:406.*
19. Mami-Chouaib, F., C. Miossec, P. Del Porto, C. Flament, F. Triebel, and T. Hercend. 1990. T cell target 1 (TCT.1): a novel target molecule for human non-major histocompatibility complex-restricted T lymphocytes. *J. Exp. Med. 172:1071.*
20. Melero, I., A. Salmeron, M. A. Balboa, J. Aramburu, and M. López-Botet. 1994. Tyrosine kinase-dependent activation of human NK cell functions upon stimulation through a 58-kDa surface antigen selectively expressed on discrete subsets of NK cells and T lymphocytes. *J. Immunol. 152:1662.*
21. Wagtmann, N., S. Rajagopalan, C. C. Winter, M. Peruzzi, and E. O. Long. 1995. Killer cell inhibitory receptors specific for HLA-C and HLA-B identified by direct binding and by functional transfer. *Immunity 3:801.*
22. Billadeau, D. D., K. M. Brumbaugh, C. J. Dick, R. A. Schoon, X. R. Bustelo, and P. J. Leibson. 1998. The Vav-Rac-1 pathway in cytotoxic lymphocytes regulates the generation of cell-mediated killing. *J. Exp. Med. 188:549.*
23. 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 SAP. *J. Immunol. 162:6981.*
24. Carretero, M., G. Palmieri, M. Llano, V. Tullio, A. Santoni, D. E. Geraghty, and M. López-Botet. 1998. Specific engagement of the CD94/NKG2-A killer inhibitory receptor by the HLA-E class Ib molecule induces SHP-1 phosphatase recruitment to tyrosine-phosphorylated NKG2-A: evidence for receptor function in heterologous transfectants. *Eur. J. Immunol. 28:1280.*
25. Tangye, S. G., J. H. Phillips, and L. L. Lanier. 2000. The CD2-subset of the Ig superfamily of cell surface molecules: receptor-ligand pairs expressed by NK cells and other immune cells. *Semin. Immunol. 12:149.*
26. Winter, C. C., and E. O. Long. 1997. A single amino acid in the p58 killer cell inhibitory receptor controls the ability of natural killer cells to discriminate between the two groups of HLA-C allotypes. *J. Immunol. 158:4026.*
27. Burshtyn, D. N., A. M. Scharenberg, N. Wagtmann, S. Rajagopalan, K. Berrada, T. Yi, J. P. Kinet, and E. O. Long. 1996. Recruitment of tyrosine phosphatase HCP by the killer cell inhibitor receptor. *Immunity 4:77.*
28. Lorenz, U., K. S. Ravichandran, D. Pei, C. T. Walsh, S. J. Burakoff, and B. G. Neel. 1994. Lck-dependent tyrosyl phosphorylation of the phosphotyrosine phosphatase SH-PTP1 in murine T cells. *Mol. Cell. Biol. 14:1824.*
29. Valiante, N. M., M. Uhrberg, H. G. Shilling, K. Lienert-Weidenbach, K. L. Arnett, A. D'Andrea, J. H. Phillips, L. L. Lanier, and P. Parham. 1997. Functionally and structurally distinct NK cell receptor repertoires in the peripheral blood of two human donors. *Immunity 7:739.*
30. Brooks, A. G., F. Borrego, P. E. Posch, A. Patamawenu, C. J. Scorzelli, M. Ulbrecht, E. H. Weiss, and J. E. Coligan. 1999. Specific recognition of HLA-E, but not classical, HLA class I molecules by soluble CD94/NKG2A and NK cells. *J. Immunol. 162:305.*
31. Eriksson, M., G. Leitz, E. Fällman, O. Axner, J. C. Ryan, M. C. Nakamura, and C. L. Sentman. 1999. Inhibitory receptors alter natural killer cell interactions with target cells yet allow simultaneous killing of susceptible targets. *J. Exp. Med. 190:1005.*