Commentary

Turning On Natural Killer Cells

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NK cells preferentially recognize and kill cells that lack expression of MHC class I (1). While inhibitory receptors expressing immunoreceptor tyrosine-based inhibition motifs prevent NK cells from harming tissues expressing normal levels of classical or nonclassical MHC class I (for a review, see reference 2), what turns on NK cells? Recent reports (3, 4), including Smith et al. in this issue (4), are beginning to unfold the receptors and signaling pathways involved in positive activation of NK cells.

Unlike T cells or B cells, which recognize antigen using clonally restricted receptors generated by gene rearrangement, NK cells appear to use a variety of different, nonrearranging receptors to initiate cytolytic activity and cytokine production (for a review, see reference 5). Many of these activating receptors are not restricted to NK cells; most are also present on T cells, and these serve as costimulatory or adhesion receptors in both cell types. Like the ability of antibodies against CD3 or the T cell antigen receptor to trigger killing or cytokine production by cytotoxic T cells, a productive strategy to search for activating NK cell receptors has involved screening for mAbs that initiate NK cell killing of Fc receptor–bearing tumor cell lines. Using this approach, several membrane receptors have been implicated in NK cell activation, including CD2 (6), CD16 (7), Ly6 (8), CD44 (9, 10), CD69 (11), NKR-P1 (8, 12), 2B4 (13–15), DNAM-1 (16), NKG2D (17), CD94/NKG2C (18), NKp44 (19), NKp46 (20), and NKp30 (3). The ligands for some of these receptors are known (e.g., CD2 for CD58 [21], 2B4 for CD48 [22], CD16 for IgG [23, 24], NKG2D for MICA [17], and CD94/NKG2C for HLA-E [25]). Expression of the ligands on target cells can initiate NK cell lysis or cytokine production, provided the NK cells are not turned off by inhibitory NK receptors recognizing class I molecules on the targets.

Although several different activating NK cell receptors have been identified, many share a common adaptor molecule or signaling pathway. Recently, Pende et al. (3) reported that NKp30 lacks known signaling motifs in its cytoplasmic domain, but noncovalently associates with CD3ζ to provide for cellular activation. CD16 also associates with CD3ζ (26), or the structurally similar FcεRIγ chain (27, 28), and this receptor complex mediates antibody-dependent cellular cytotoxicity. NKp46 (29) and mouse NKR-P1 (30) are other NK cell receptors linked to CD3ζ or FcεRIγ, respectively. Although not physically associated, CD3ζ has been implicated in CD2-mediated activation of NK cells (31). NK cells express both CD3ζ and FcεRIγ, and these adaptor molecules form disulfide-bonded homodimers, as well as heterodimers with each other (28). CD3ζ and FcεRIγ have immunoreceptor tyrosine-based activation motifs (ITAMs) in their cytoplasmic domains that upon phosphorylation caused by receptor ligation recruit the cytoplasmic tyrosine kinases Syk and ZAP70 (both of which are expressed in NK cells; for a review, see reference 32). Ligands for NKp30, NKp46, and NKR-P1 have not been identified; however, there are suggestions that these receptors are involved in NK cell recognition of certain tumors (3, 19, 33).

Other activating NK cell receptors signal by association with another ITAM-containing adaptor protein, DAP12. Like CD3ζ, DAP12 has a very short extracellular domain (of ~14 amino acids) and is expressed as a disulfide-bonded homodimer on the surface of NK cells, myeloid cells, and a subset of T cells (34, 35). Whereas CD3ζ has three ITAMs, DAP12 and FcεRIγ each have a single ITAM. DAP12 associates with the activating isoforms of the human killer cell Ig-like receptors (KIRs) (34), mouse Ly49D and Ly49H (36, 37), the CD94/NKG2C receptor (38), and NKp44 (39). In this issue, Smith et al. (4) describe an mAb against Ly49H and demonstrate that cross-linking this receptor results in the activation of a subset of mouse NK cells. Known ligands of these DAP12-associated receptors include HLA-C for KIR, HLA-E for CD94/NKG2C, and H-2D^d for Ly49D (to date, the ligand for Ly49H has not been identified). As with CD3ζ or FcεRIγ, ligation of these DAP12-associated receptors results in tyrosine phosphorylation of the ITAM of DAP12 and recruitment of Syk or ZAP70 (34, 40), thereby initiating cytolytic activity and cytokine production. DAP12, CD3ζ, and FcεRIγ all

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possess an acidic amino acid (aspartic acid) in their transmembrane domains that provides for their association with charged amino acids in the transmembrane domains of the ligand-binding receptor subunits. While CD3ζ and FcεRIγ may be interchangeable in their association with certain partners, DAP12 interacts with completely distinct glycoproteins, indicating exquisite specificity in these multisubunit receptor complexes.

NK cell development and cytolytic function are intact in mice or humans with disrupted ZAP70 (41, 42) or Syk (43) genes, suggesting the existence of receptors using other signaling pathways. Activating NK cell receptors not linked to ITAM-containing adaptor proteins have been identified. The activating NK cell receptor 2B4 (13, 14, 44) uses a signaling pathway involving the cytoplasmic tyrosine phosphatase SHP-2 and the Src homology 2 (SH2)-containing intracellular adaptor protein SAP (15). SAP, identified by its association with the T cell costimulatory molecule SLAM (45), likely serves a common role for several receptors in NK and T cells. NKG2D, an activating receptor on T and NK cells for the nonclassical MICA and MICB antigens (17), uses the DAP10 adaptor protein for signal transduction (46). DAP10 is a membrane-anchored, disulfide-linked homodimer with an acidic amino acid (aspartic acid) in the transmembrane that permits association with a basic amino acid (arginine) in the transmembrane of NKG2D (46). Phosphorylation of the tyrosine residue in the YxxM motif in the DAP10 cytoplasmic domain recruits the p85 subunit of phosphatidylinositol 3-kinase (PI3-kinase) (46). Inhibitors of PI3-kinase prevent NKG2D-mediated NK cell cytotoxicity and cytokine production, implicating this signaling pathway in DAP10-induced activation. Expression of DAP10 in myeloid cells, which lack NKG2D, predicts the existence of other receptors that will use DAP10 for signal transduction (46).

Although many receptors are now being implicated in NK cell activation, it is uncertain whether any individual receptor alone will be sufficient to initiate effector function. Rather, additive or synergistic interactions between multiple receptors may be necessary to stimulate cytotoxicity and cytokine production. Experimental findings support the latter. In the recent studies of Pende et al. (3), the mAb against NKp30 alone only marginally blocked NK cell killing of several tumors. However, more dramatic effects were observed when blocking antibodies against several NK cell receptors and adhesion molecules were used in combination. Thus, the emerging concept is that NK cell function is determined by the integration of signals from several activating receptors that are regulated by the inhibitory receptors for MHC class I, based on the density and array of ligands and class I molecules expressed by the antigen-presenting cell. Infection and inflammation are often accompanied by the release of local cytokines (e.g., IL-15, TNF-α, type I IFNs, chemokines). These cytokines may serve not only to activate NK cells, but also to upregulate ligands (e.g., the intercellular adhesion molecules [ICAMs], CD48, CD58) for adhesion or costimulatory receptors that are present on NK cells. In this environment, rich in stimulatory cytokines and ligands for activating receptors, NK cells may provide a critical bridge between the innate and adaptive immune systems.

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References

1. Karre, K., H.G. Ljunggren, G. Piontek, and R. Kiessling. 1986. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defense strategy. *Nature*. 319: 675–678.
2. Long, E.O. 1999. Regulation of immune responses through inhibitory receptors. *Annu. Rev. Immunol.* 17:875–904.
3. Pende, D., S. Parolini, A. Pessino, S. Sivori, R. Augugliaro, L. Morelli, E. Marcenaro, L. Accame, A. Malaspina, R. Biassoni, et al. 1999. Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. *J. Exp. Med.* 190:1505–1516.
4. Smith, H.R.C., H.C. Chuang, L.L. Wang, M. Salcedo, J.W. Heusel, and W.M. Yokoyama. 2000. Nonstochastic coexpression of activation receptors on murine natural killer cells. *J. Exp. Med.* 191:1341–1354.
5. Lanier, L.L. 1998. NK cell receptors. *Annu. Rev. Immunol.* 16:359–393.
6. Siliciano, R.F., J.C. Pratt, R.E. Schmidt, J. Ritz, and E.L. Reinherz. 1985. Activation of cytolytic T lymphocyte and natural killer cell function through the T11 sheep erythrocyte binding protein. *Nature*. 317:428–430.
7. Lanier, L.L., J.J. Ruitenberg, and J.H. Phillips. 1988. Functional and biochemical analysis of CD16 antigen on natural killer cells and granulocytes. *J. Immunol.* 141:3478–3485.
8. Karlhofer, F.M., and W.M. Yokoyama. 1991. Stimulation of murine natural killer (NK) cells by a monoclonal antibody specific for the NK1.1 antigen. IL-2-activated NK cells possess additional specific stimulation pathways. *J. Immunol.* 146: 3662–3673.
9. Galandrini, R., R. De Maria, M. Piccoli, L. Frati, and A. Santoni. 1994. CD44 triggering enhances human NK cell cytotoxic functions. *J. Immunol.* 153:4399–4407.
10. Sconocchia, G., J.A. Titus, and D.M. Segal. 1994. CD44 is a cytotoxic triggering molecule in human peripheral blood NK cells. *J. Immunol.* 153:5473–5481.
11. Moretta, A., A. Poggi, D. Pende, G. Tripodi, A.M. Orengo, N. Pella, R. Augugliaro, C. Bottino, E. Ciccone, and L. Moretta. 1991. CD69-mediated pathway of lymphocyte activation: anti-CD69 monoclonal antibodies trigger the cytolytic activity of different lymphoid effector cells with the exception of cytolytic T lymphocytes expressing T cell receptor α/β. *J. Exp. Med.* 174:1393–1398.
12. Chambers, W.H., N.L. Vujanovic, A.B. DeLeo, M.W. Olszowy, R.B. Herberman, and J.C. Hiserodt. 1989. Monoclonal antibody to a triggering structure expressed on rat natural killer cells and adherent lymphokine-activated killer cells. *J. Exp. Med.* 169:1373–1389.
13. Garni-Wagner, B.A., A. Purohit, P.A. Mathew, M. Bennett, and K. Kumar. 1993. A novel function-associated molecule related to non-MHC-restricted cytotoxicity mediated by activated natural killer cells and T cells. *J. Immunol.* 151:60–70.
14. Valiante, N.M., and G. Trinchieri. 1993. Identification of a novel signal transduction surface molecule on human cyto-

toxic lymphocytes. *J. Exp. Med.* 178:1397–1406.
15. Tangye, S.G., S. Lazetic, E. Woollatt, G.R. Sutherland, L.L. Lanier, and J.H. Phillips. 1999. Human 2B4, an activating NK cell receptor, recruits the protein tyrosine phosphatase SHP-2 and the adaptor signaling protein SAP. *J. Immunol.* 162:6981–6985.
16. Shibuya, A., D. Campbell, C. Hannum, H. Yssel, K. Franz-Bacon, T. McClanahan, T. Kitamura, J. Nicholl, G.R. Sutherland, L.L. Lanier, and J.H. Phillips. 1996. DNAM-1, a novel adhesion molecule involved in the cytolytic function of T lymphocytes. *Immunity.* 4:573–581.
17. Bauer, S., V. Groh, J. Wu, A. Steinle, J.H. Phillips, L.L. Lanier, and T. Spies. 1999. Activation of natural killer cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science.* 285:727–730.
18. Cantoni, C., R. Biassoni, D. Pende, S. Sivori, L. Accame, L. Pareti, G. Semenzato, L. Moretta, A. Moretta, and C. Bottino. 1998. The activating form of CD94 receptor complex: CD94 covalently associates with the Kp39 protein that represents the product of the NKG2-C gene. *Eur. J. Immunol.* 28: 327–338.
19. Vitale, M., C. Bottino, S. Sivori, L. Sanseverino, R. Castriconi, E. Marcenaro, R. Augugliaro, L. Moretta, and A. Moretta. 1998. NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex–restricted tumor cell lysis. *J. Exp. Med.* 187:2065–2072.
20. Sivori, S., M. Vitale, L. Morelli, L. Sanseverino, R. Augugliaro, C. Bottino, L. Moretta, and A. Moretta. 1997. p46, a novel natural killer cell–specific surface molecule that mediates cell activation. *J. Exp. Med.* 186:1129–1136.
21. Dustin, M.L., M.E. Sanders, S. Shaw, and T.A. Springer. 1987. Purified lymphocyte function-associated antigen 3 binds to CD2 and mediates T lymphocyte adhesion. *J. Exp. Med.* 165:677–692.
22. Brown, M.H., K. Boles, P. Anton van der Merwe, V. Kumar, P.A. Mathew, and N.A. Barclay. 1998. 2B4, the natural killer and T cell immunoglobulin superfamily surface protein, is a ligand for CD48. *J. Exp. Med.* 188:2083–2090.
23. Perussia, B., S. Starr, S. Abraham, V. Fanning, and G. Trinchieri. 1983. Human natural killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. I. Characterization of the lymphocyte subset reactive with B73.1. *J. Immunol.* 130:2133–2141.
24. Lanier, L.L., A.M. Le, J.H. Phillips, N.L. Warner, and G.F. Babcock. 1983. Subpopulations of human natural killer cells defined by expression of the Leu-7 (HNK-1) and Leu-11 (NK-15) antigens. *J. Immunol.* 131:1789–1796.
25. Braud, V.M., D.S.J. Allan, C.A. O'Callaghan, K. Soderstrom, A. D'Andrea, G.S. Ogg, S. Lazetic, N.T. Young, J.I. Bell, J.H. Phillips, L.L. Lanier, and A.J. McMichael. 1998. HLA-E binds to natural killer cell receptors CD94/NKG2A, B, and C. *Nature.* 391:795–798.
26. Lanier, L.L., G. Yu, and J.H. Phillips. 1989. Co-association of CD3ζ with a receptor (CD16) for IgG Fc on human natural killer cells. *Nature.* 342:803–805.
27. Wirthmueller, U., T. Kurosaki, M.S. Murakami, and J.V. Ravetch. 1992. Signal transduction by FcγRIII (CD16) is mediated through the γ chain. *J. Exp. Med.* 175:1381–1390.
28. Lanier, L.L., G. Yu, and J.H. Phillips. 1991. Analysis of FcγRIII (CD16) membrane expression and association with CD3ζ and FcεRI-γ by site-directed mutation. *J. Immunol.* 146:1571–1576.
29. Pessino, A., S. Sivori, C. Bottino, A. Malaspina, L. Morelli, L. Moretta, R. Biassoni, and A. Moretta. 1998. Molecular cloning of NKp46: a novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity. *J. Exp. Med.* 188:953–960.
30. Arase, N., H. Arase, S.Y. Park, H. Ohno, C. Ra, and T. Saito. 1997. Association with FcRγ is essential for activation signal through NKR-P1 (CD161) in natural killer (NK) cells and NK1.1$^+$ T cells. *J. Exp. Med.* 186:1957–1963.
31. Vivier, E., P.M. Morin, C. O'Brien, S.F. Schlossman, and P. Anderson. 1991. CD2 is functionally linked to the ζ-natural killer receptor complex. *Eur. J. Immunol.* 21:1077–1080.
32. Chan, A.C., D.M. Desai, and A. Weiss. 1994. The role of protein tyrosine kinases and protein tyrosine phosphatases in T cell antigen receptor signal transduction. *Annu. Rev. Immunol.* 12:555–592.
33. Ryan, J.C., E.C. Niemi, M.C. Nakamura, and W.E. Seaman. 1995. NKR-P1A is a target-specific receptor that activates natural killer cell cytotoxicity. *J. Exp. Med.* 181:1911–1915.
34. Lanier, L.L., B.C. Corliss, J. Wu, C. Leong, and J.H. Phillips. 1998. Immunoreceptor DAP12 bearing a tyrosine-based activation motif is involved in activating NK cells. *Nature.* 391: 703–707.
35. Tomasello, E., L. Olcese, F. Vely, C. Geourgeon, M. Blery, A. Moqrich, D. Gautheret, M. Djabali, M.-G. Mattei, and E. Vivier. 1998. Gene structure, expression pattern, and biological activity of mouse killer cell activating receptor-associated protein (KARAP)/DAP-12. *J. Biol. Chem.* 273:34115–34119.
36. Smith, K.M., J. Wu, A.B.H. Bakker, J.H. Phillips, and L.L. Lanier. 1998. Ly49D and Ly49H associate with mouse DAP12 and form activating receptors. *J. Immunol.* 161:7–10.
37. Gosselin, P., L.H. Mason, J. Willette-Brown, J.R. Ortaldo, D.W. McVicar, and S.K. Anderson. 1999. Induction of DAP12 phosphorylation, calcium mobilization, and cytokine secretion by Ly49H. *J. Leukoc. Biol.* 66:165–171.
38. Lanier, L.L., B. Corliss, J. Wu, and J.H. Phillips. 1998. Association of DAP12 with activating CD94/NKG2C NK cell receptors. *Immunity.* 8:693–701.
39. Cantoni, C., C. Bottino, M. Vitale, A. Pessino, R. Augugliaro, A. Malaspina, S. Parolini, L. Moretta, A. Moretta, and R. Biassoni. 1999. NKp44, a triggering receptor involved in tumor cell lysis by activated human natural killer cells, is a novel member of the immunoglobulin superfamily. *J. Exp. Med.* 189:787–796.
40. McVicar, D.W., L.S. Taylor, P. Gosselin, J. Willette-Brown, A.I. Mikhael, R.L. Geahlen, M.C. Nakamura, P. Linnemeyer, W.E. Seaman, S.K. Anderson, et al. 1998. DAP12-mediated signal transduction in natural killer cells. A dominant role for the syk protein-tyrosine kinase. *J. Biol. Chem.* 273:32934–32942.
41. Elder, M.E., D. Lin, J. Clever, A.C. Chan, T.J. Hope, A. Weiss, and T.G. Parslow. 1994. Human severe combined immunodeficiency due to a defect in ZAP-70, a T cell tyrosine kinase. *Science.* 264:1596–1599.
42. Negishi, I., N. Motoyama, K.-i. Nakayama, K. Nakayama, S. Senju, S. Hatakeyama, Q. Zhang, A.C. Chan, and D.Y. Loh. 1995. Essential role for ZAP-70 in both positive and negative selection of thymocytes. *Nature.* 376:435–438.
43. Colucci, F., M. Turner, E. Schweighoffer, D. Guy-Grand, V. Di Bartolo, M. Salcedo, V.L. Tybulewicz, and J.P. DiSanto. 1999. Redundant role of the Syk protein tyrosine

kinase in mouse NK cell differentiation. *J. Immunol.* 163: 1769–1774.
44. 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: 1676–1683.
45. 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*. 395:462–469.
46. Wu, J., Y. Song, A.B.H. Bakker, S. Bauer, V. Groh, T. Spies, L.L. Lanier, and J.H. Phillips. 1999. An activating receptor complex on natural killer and T cells formed by NKG2D and DAP10. *Science*. 285:730–732.