Commentary

What Goes Up Must Come Down: The Emerging Spectrum of Inhibitory Receptors

By Wayne M. Yokoyama

From the Howard Hughes Medical Institute, Rheumatology Division, Washington University School of Medicine, St. Louis, Missouri 63110

For much of the last few decades, investigators have concentrated on delineating the parameters that involve the direct activation of the immune system. In general, receptor engagement, by antigen, cytokine, or counterreceptor, initiates a cascade of distinct biochemical events in lymphocytes and other immune cells, leading to downstream effector functions. Concurrent engagement of other (costimulatory) receptors tends to enhance these responses. Inasmuch as these pathways have been described in great detail, strategies to alter immune responses, such as to arrest overactive autoimmune states, to enhance immune responses to tumors or pathogens, or to provoke immunological memory from vaccination have been focused on modulation of these activation processes.

However, recent evidence clearly demonstrates that ligation of another functional class of immune receptors leads instead to inhibition of activation pathways (for review see Parham, P., ed. 1997. NK cells, MHC Class I Antigens, and Missing Self. *Immunol. Rev.* 155:1–221.). Important information is emerging on the nature of such inhibition, providing a glimpse into the complexity of the ligand specificities and biochemical mechanisms of inhibitory receptors. In this issue of the *Journal*, the Colonna laboratory together with the López-Botet group (2) illustrate new concepts concerning inhibition. To provide a context in which to appreciate this study as well as a panoply of related investigations recently published by other groups, it is useful to review the current status of this rapidly moving field and discuss the myriad new scientific issues that are raised.

The paradigm of inhibitory receptors can be illustrated by studies from a variety of experimental systems. For example, previous analyses demonstrated that cellular targets expressing certain MHC class I molecules are protected from killing by NK cells, consistent with the so-called "missing-self" hypothesis (3). Absence of target cell MHC class I expression releases NK cells from inhibition, permitting activation of natural killing. Other explanations were initially favored, such as the blockade of NK cell activation receptors by MHC class I ("target interference"), an example that highlights the previous conceptual framework for an immune system involving exclusively activation-type receptors. However, it is now known that inhibition is mediated by engagement of distinct NK cell receptors specific for MHC class I (4), leading to biochemical events, i.e., "negative signals," that globally inhibit NK cell function (5). Similarly, ligation of the B cell antigen receptor with whole anti-Ig has long been associated with failure to activate, in contrast to stimulation with $F(ab')_2$ fragments of anti-Ig. The effect of whole anti-Ig has now been demonstrated to be due to simultaneous engagement of one type of Fc receptor (FcγRIIb1) on the B cell. FcγRIIb1 is then phosphorylated on tyrosine residues in a consensus sequence (I/VxYxxL), otherwise known as the immunoreceptor tyrosine-based inhibitory motif (ITIM; references 6 and 7). Although there is presumably a tyrosine kinase involved in phosphorylation of the ITIM, whether a specific kinase is required is not yet known for B cells. Nevertheless, the phosphorylated inhibitory receptor can then recruit intracellular phosphatases (SHP-1 or SHIP, see below) which likely dephosphorylate second messenger molecules in activation pathways. Comparable involvement of the CD22 receptor during B cell antigen receptor (BCR) stimulation also results in functional inactivation (8).

With respect to the MHC class I–specific NK cell receptors, a biochemical mechanism very similar to the ITIM system of FcγRIIb1 operates despite a large number of different receptors. The inhibitory NK cell receptors can be divided into two general structural types (1, 9). One is illustrated by the mouse Ly-49 family of molecules and human CD94/NKG2 receptors, which are type II integral membrane proteins belonging to the C-type lectin superfamily (10, 11). These receptors are expressed as disulfide-linked dimers, either as homodimers, such as Ly-49 molecules (10), or heterodimers, such as the CD94/NKG2 receptors (11, 12). The other type of receptor was first characterized on human NK cells, collectively termed killer inhibitory receptors (KIR). These are type I integral membrane proteins belonging to the Ig superfamily and are homologous to the bovine Fcγ2 and human Fcα receptors (13, 14). Although initially there was speculation that mice and humans use structurally distinct receptors to subserve the same function, recent studies have indicated that both structural types are expressed on NK cells from both species (11, 12, 15, 16). At the moment, it is not known why both structural types are necessary as indicated by their conservation in evolution. The ever-increasing repertoire of NK cell receptors suggests the possibility that individual receptors may work in concert with the others. Perhaps the receptors have overlapping specificities for MHC class I ligands, or, alternatively, some of these receptors may be related to each other in the same way that CD4 and CD8 are coreceptors

J. Exp. Med. © The Rockefeller University Press • 0022-1007/97/12/1803/06 $2.00
Volume 186, Number 11, December 1, 1997 1803–1808
http://www.jem.org

for the TCR, or they may be physically associated with each other in a complex, analogous to the CD3 complex of the TCR. These issues have not yet been addressed, but answers should be forthcoming as detailed experimental systems are developed and the fine specificities of the receptors become known. Nevertheless, the two structural types of NK cell receptors appear to be complementary rather than totally distinct, as indicated by shared properties, including their negative signaling mechanisms.

Despite obvious differences, i.e., in structure and plasma membrane orientation, both types of NK cell receptors apparently use the same inhibitory mechanism involving cytoplasmic ITIMs (5, 17). Recent studies indicate that the Src family protein tyrosine kinase Lck can phosphorylate the ITIM of a KIR expressed in transfection studies of Jurkat T cells (18). Although the latter findings require extension to untransfected NK cells, and a specific kinase has not been reported with respect to phosphorylation of the lectin-like receptors, the phosphorylated ITIMs of either NK cell receptor type would then be able to recruit the intracellular tyrosine phosphatase SHP-1 in a manner reminiscent of ITIM-associated FcγRIIb1 inhibition of B cells.

There are other similarities between the two general types of NK cell receptors. Both types are expressed on small subsets of T cells that can also be inhibited. Furthermore, both types consist of families of molecules with highly related sequences. For example, members of the Ly-49 family display ~80% amino acid identity to each other (19, 20). Similarly, the KIR molecules display >80% identity in the extracellular domains (21–28). However, although most members within a family appear to be inhibitory receptors, based either on experimental analysis or the presence of cytoplasmic ITIMs in deduced sequences, there are other family members without ITIMs. Even among members of the Ly-49 family, which initially were characterized as inhibitory receptors, there are other members, such as Ly-49D, which lack ITIMs (20). Similar isoforms such as p50 have been described in the KIR family (29). Thus far, it is unclear as to whether all of the different receptor isoforms arise from alternative splicing, as recently described, or distinct genes, or both. Nevertheless, the ITIM-less molecules appear to directly activate or costimulate distal cellular processes, as recently described in the *Journal* and elsewhere (29–31).

The rules governing the expression of inhibitory versus activation isoforms have not yet been elucidated, but the functional activity of both types of receptors probably depends upon coassociation with transmembrane or cytoplasmic molecules. In this regard, it will be important to discriminate between costimulatory effects and direct activation through these isoforms. Nevertheless, it may be important that the activation isoforms of either membrane orientation have charged residues in transmembrane domains despite the absence of obvious activation motifs in their cytoplasmic domains, such as the immunoreceptor tyrosine-based activation motif (ITAM; reference 32). Of course, this could reflect unique signaling mechanisms, but perhaps the activation isoforms are associated with other molecules that are responsible for transmitting activation signals, analogous to the CD3 components of the TCR or the signaling components of BCR and FcεRI (33). The activation mechanisms used by these NK cell activation receptors are just beginning to be explored. Early characterization has demonstrated associated disulfide-linked chains (34), which may already be known or perhaps may be novel signal transduction subunits bearing ITAMs for recruitment of tyrosine kinases. As in the case of other multimeric receptors, such components may also be necessary for full expression of activation isoforms (33), and it will be of interest to determine if activation isoforms with either plasma membrane orientation use the same set of associated transmembrane subunits. In addition, although MHC class I ligands have been described for some activation isoforms (29–31), identification of ligands for the others are needed to fully appreciate their role in immune function.

For the inhibitory receptors, the ITIM may be involved in different inhibitory mechanisms, dependent on associated phosphatases. For example, in mast cells, FcγRIIb1 inhibits FcεRI signaling by the recruitment of the SH2-domain–containing inositol polyphosphate 5-phosphatase, SHIP (35). In B cells, FcγRIIb1 has been reported to recruit SHP-1 (36), but the functional importance of this association is controversial because recent studies indicate that SHP-1 is not required for FcγRIIb1-mediated inhibition (37) although it apparently is required for inhibitory NK cell receptor function (17, 38). Nevertheless, either SHP-1 or SHIP can be recruited by phosphorylated ITIMs. What is the basis for such discordant recruitment of second messengers? The difference does not appear to be due solely to differential expression of SHP-1 versus SHIP. When both phosphatases are available, transfected FcγRIIb1 cytoplasmic ITIMs recruit only SHIP, whereas transfected KIR tails recruit only SHP-1 (39, 40), implying another level of specificity, perhaps due to the contribution of other specific binding sites resulting in differences in affinities for the ITIMs, or involvement of other molecules, such as adapter proteins, which could modify the pathways.

If both SHP-1 and SHIP inhibitory mechanisms are available in the cell, are the functional consequences the same (redundancy), or are only subsets of activation events blocked, resulting in expression of certain effector functions but not others (differential inhibition or complementation)? Indeed, there are differences between outcomes that have been discovered thus far (39). First, even in the same cell, SHP-1 recruitment by transfected KIR ITIMs affects mobilization of intracellular Ca^{2+} stores, whereas SHIP recruitment by FcγRIIb1 ITIMs diminishes influx of extracellular Ca^{2+}. Second, the KIR ITIMs block apoptosis by BCR activation, whereas FcγRIIb1 engagement has no effect. However, in SHIP-deficient cells FcγRIIb1 cross-linking appears to enhance apoptosis. Therefore, divergence in downstream effector functions may result from differences in ITIM-associated signaling molecules, and there may be still other, yet to be described, processes that are specifically affected by other inhibitory receptors and associated second messengers.

Recent studies also indicate that ITIMs may be associated with other inhibitory molecules such as the Src kinase Csk and SHP-2. Csk can phosphorylate a COOH-terminal negative regulatory tyrosine residue in Src family kinases, thereby inhibiting Src protein tyrosine kinase activity and cellular activation (41). Engagement of FcγRIIb1 results in a direct association between Csk and a RasGTPase-activating phosphoprotein, suggesting that both of these molecules may play a role in inhibition (42). However, the position of these molecules in the inhibitory pathway is not known as yet, nor is it known whether either molecule could directly associate with the ITIMs. Another tyrosine phosphatase, SHP-2, can bind the phosphorylated ITIM, but its functional role is less well understood (43). SHP-2 associates with a family of proteins, termed signal-regulatory proteins, which appear to have negative regulatory effects (44). The detailed roles of these molecules are less well known, but their large number suggests additional complexity. Therefore, in spite of major advances, there is still much more to be learned about the mechanisms induced by inhibitory receptors; many of these insights will come from better appreciation of the role of associated molecules and their relationships in the inhibitory pathways.

The genes encoding both structural types of NK cell receptors are genetically clustered. The lectin-like (both activation and inhibitory isoforms) receptors are encoded in a large (>2 megabases in mice) chromosomal region (mouse chromosome 6, human chromosome 12p13; reference 45) termed the NK gene complex (NKC) that contains several gene families for receptors that are (thus far) primarily expressed on NK cells (and on a small subset of T cells, particularly the so-called NK/T cells). Encoded within the NKC are NKR-P1, Ly-49, CD94, NKG2, and CD69. Although CD69 is more broadly expressed, all are type II membrane proteins, C-type lectins, and disulfide-linked dimers. There appears to be significant allelic polymorphism for at least some of the genes. In contrast, the KIR molecules are encoded in another genetic region (proximal mouse chromosome 7, human 19q13.4; reference 46) with less allelic polymorphism. In addition, KIR molecules are related in sequence to a growing list, including the previously identified mouse gp49 (47), and the newly cloned immunoglobulin-like transcript 1 (ILT1; reference 48), ILT3 (49), ILT4, ILT5 (48), ILT2/leukocyte immunoglobulin-like receptor 1 (LIR-1; references 48 and 50), leukocyte-associated immunoglobulin-like receptor 1 (LAIR-1; reference 51), paired Ig-like receptors (PIR)-A and -B (52), p91 (53), and monocyte immunoglobulin-like receptor (MIR; reference 46). The genes for many of these molecules appear to be physically linked in the same genetic complex as the KIR genes (46). This complex may encode additional receptors with similar properties, including general overall structure with or without ITIMs. However, some of the known molecules have more (up to six; references 52 and 53) or less (one; reference 51) Ig-like domains and have broader tissue distribution than just on NK cells, as their names imply. Furthermore, gp49B1 has been shown to inhibit FcεR1-mediated mast cell activation (54), and ILT3 has been reported to inhibit antigen capture and processing in APCs (49), demonstrating the inhibitory influence of these receptors beyond NK and B cells.

Certainly, it is not surprising that inhibitory receptors are broadly distributed, but currently it is difficult to solidify support for a unifying theme explaining the basis for their expression. However, inhibitory receptors may be distributed according to the activation pathways that are used in a given cell rather than strictly according to cell lineage. For example, any cell that uses activation systems involving the recruitment and activation of tyrosine kinases may require inhibitory pathways employing tyrosine phosphatases to modify the initiation, extent, and/or length of activation. With the recognition that inhibition can also occur with SHIP, cells with activation pathways involving inositol phosphate metabolism should also require SHIP-type inhibitory pathways, and so on. As more is recognized about the mechanisms of inhibitory pathways, more sense will be made of the broad distribution of inhibiting receptors. It is possible that related receptors will be described on other cells, including nonhematopoietic cells, as more are identified by molecular cloning and/or detailed analysis of the corresponding genetic regions. Nevertheless, the expression of these molecules on such a diversity of immune cells combined with previous functional information strongly suggests that inhibitory receptors influence many more cells and processes than was initially recognized.

In this issue, Colonna et al. examine one such inhibitory receptor, ILT2 (2). Although it has only 40% amino acid identity to KIR molecules, ILT2 binds MHC class I molecules. Not surprisingly, engagement of ILT2 leads to inhibition, since it contains four putative ITIMs, and phosphorylation of the ITIMs with pervanadate leads to SHP-1 association. In addition, Cosman et al. have recently shown that this same receptor (termed LIR-1), binds the cytomegalovirus UL18 gene product, an MHC class I–like molecule (50). Also, UL18 has been reported to engage other NK cell receptors, including the lectin-like receptor CD94 (55), resulting in inhibition of NK cell activity, presumably to avoid NK cell–mediated viral clearance. Although these data strongly suggest a viral immune evasion strategy in which UL18 can bind multiple inhibitory receptors, thereby globally inhibiting NK cell activity through different receptors, these findings have broader implications. ILT2/LIR-1 and newly described related molecules are not only expressed on NK cells and subsets of T cells, but also on B cells, mast cells, macrophages, and dendritic cells (2, 50). ILT2/LIR-1 inhibition of activation is notable because it can also occur in basophils (rat basophil leukemic cells) stimulated by FcεRI cross-linking, in T cells stimulated by superantigen, in B cells activated by anti-Ig, and in monocytes and dendritic cells stimulated through HLA-DR (2). Although the assays frequently involved cross-linking with anti–ILT2/LIR-1 antibodies rather than "physiologic" ligands, or UL18, the data suggest that the biochemical events in these activation pathways must be similar to be susceptible to a single inhibitory receptor. On the other hand, it is possible that a different subset of the four ITIMs

is required in each cell type due to differential recruitment of inhibitory second messengers; it is not yet clear why some of the inhibitory receptors have multiple ITIMs whereas others have only one. Nevertheless, the inhibition of a broad array of stimuli is consistent with a viral strategy to globally block the immune system, and not just affect NK cells.

Another surprising outcome from the studies of Colonna et al. (2) and Cosman et al. (50) is that MHC class I–specific receptors are expressed on cells for which MHC class I–associated inhibition has not been previously described. Although the basophil studies involved ILT2-transfected RBL cells, the other cells investigated (NK, T, and B) constitutively expressed ILT2, reflecting the broad distribution of this molecule. Perhaps the missing-self phenomenon was previously missed on these cells and is much more broadly applicable than just to NK cells. Such receptors may also have MHC class II or MHC class Ib ligands since these counterreceptors are structurally related, broadening the possibilities.

It is also possible that the putative inhibitory receptors may have other ligands, such as soluble factors, i.e., cytokines or hormones. Indeed, recent studies by Fan et al. (56) have indicated that KIR molecules are structurally related to receptors for hematopoietic factors and hormones such as erythropoietin, prolactin, and growth hormone. Consistent with sequence analysis and site-directed mutagenesis studies (57), a three-dimensional model for one of the KIR molecules reveals two Ig-like domains containing two antiparallel β-sheets arranged at a 60° angle. The joining "elbow" segment between the domains contains residues that determine KIR specificity for MHC class I, suggesting that such residues are contact sites. By analogy to the soluble factor receptors that dimerize upon ligand engagement, KIR molecules may bind two distinct sites on MHC class I, resulting in KIR dimerization. Although the "footprint" of KIR binding on MHC class I localizes to the MHC class I α1 helix (58), there may also be other, possibly nonpolymorphic, sites on MHC class I, since previous studies of contact sites have focused on determining specificity for allelic determinants (59, 60). Alternatively, one of the KIR molecules may be a relatively invariant receptor chain in a multimeric complex. The three-dimensional structure also suggests that KIR-related molecules may bind soluble ligands. Those molecules with several Ig domains may have multiple contact sites for their ligands, or they may bind multiple ligands. Further analysis of ligand specificity is extremely important to fully understand the physiologic importance of these putative receptors.

Nevertheless, the inhibitory receptors already provide new targets for therapeutic alteration of the immune system to prevent or treat disease. In this regard, perhaps we can take a clue from viral evasion strategies to target ubiquitously expressed inhibitory receptors. The recently described inhibitory receptor LAIR-1, with one Ig-like domain (51), may be especially interesting here. It contains ITIMs, can recruit SHP-1 and SHP-2, and can inhibit NK cell activity. However, according to flow cytometry with an anti–LAIR-1–specific mAb, the receptor is apparently expressed by the vast majority of B, T, and NK lymphocytes and monocytes, providing a means to simultaneously target a large number of immune cells by focusing on a single receptor.

Interestingly, however, any therapeutic maneuvers involving inhibitory receptors obviously will have consequences that are opposite to either interrupting or increasing the function of activation receptors as reported to date. Assuming that there is no activation isoform of LAIR-1, a therapeutic receptor engagement, i.e., with an mAb or a pharmaceutical mimic of its physiologic ligand, will likely be capable of inhibiting the functions of a broad array of immune cells, thereby offering a potential new form of therapy for autoimmune diseases. On the other hand, approaches to interrupt inhibitory receptor function at either the ligand binding or biochemical signaling steps have the prospect of enhancing immune responses, providing the basis for an exciting new armamentarium of immune modulating drugs.

I thank my colleagues Lewis Lanier, David Cosman, Eric Long, and Marco Colonna for sharing information before publication, Miguel Lopez-Bótet for insightful discussions, and Andy Chan, Matt Thomas, and Larry Wang for their critical evaluation of this manuscript.

Research in the Yokoyama laboratory is supported by grants from the National Institutes of Health, the Monsanto/Searle-Washington University Research Agreement, and the Barnes-Jewish Hospital Research Foundation. W.M. Yokoyama is an investigator of the Howard Hughes Medical Institute.

Address correspondence to Dr. Wayne M. Yokoyama, Howard Hughes Medical Institute, Rheumatology Division, Box 8045, Washington University Medical Center, 660 South Euclid Avenue, St. Louis, MO 63110. E-mail: yokoyama@imgate.wustl.edu

Received for publication 10 September 1997 and in revised form 30 September 1997.

References

1. Deleted in proof.
2. Colonna, M., F. Navarro, T. Bellon, M. Llano, P. Garcia, J. Samaridis, L. Angman, M. Cella, and M. López-Botet. 1997. A common inhibitory receptor for MHC class I molecules on human lymphoid and myelomonocytic cells. *J. Exp. Med.* 186:1809–1818.
3. Kärre, K. 1985. Role of target histocompatibility antigens in regulation of natural killer activity: a reevaluation and a hy-

pothesis. *In* Mechanisms of Cytotoxicity by NK Cells. R.B. Herberman and D.M. Callewaert, editors. Academic Press, Orlando, FL. 81–103.
4. Karlhofer, F.M., R.K. Ribaudo, and W.M. Yokoyama. 1992. MHC class I alloantigen specificity of Ly-49$^+$ IL-2-activated natural killer cells [see comments]. *Nature (Lond.)*. 358:66–70.
5. 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*. 4:77–85.
6. Muta, T., T. Kurosaki, Z. Misulovin, M. Sanchez, M.C. Nussenzweig, and J.V. Ravetch. 1994. A 13-amino-acid motif in the cytoplasmic domain of FcγRIIB modulates B-cell receptor signalling. *Nature (Lond.)*. 368:70–73 (see erratum *Nature (Lond.)*. 1994. 369:340).
7. Thomas, M.L. 1995. Of ITAMs and ITIMs: turning on and off the B cell antigen receptor. *J. Exp. Med*. 181:1953–1956.
8. Doody, G.M., L.B. Justement, C.C. Delibrias, R.J. Matthews, J. Lin, M.L. Thomas, and D.T. Fearon. 1995. A role in B cell activation for CD22 and the protein tyrosine phosphatase SHP. *Science (Wash. DC)*. 269:242–244.
9. Yokoyama, W.M. 1995. Natural killer cells. Right-side-up and up-side-down NK-cell receptors. *Curr. Biol*. 5:982–985.
10. Yokoyama, W.M., L.B. Jacobs, O. Kanagawa, E.M. Shevach, and D.I. Cohen. 1989. A murine T lymphocyte antigen belongs to a supergene family of type II integral membrane proteins. *J. Immunol*. 143:1379–1386.
11. Lazetic, S., C. Chang, J.P. Houchins, L.L. Lanier, and J.H. Phillips. 1996. Human natural killer cell receptors involved in MHC class I recognition are disulfide-linked heterodimers of CD94 and NKG2 subunits. *J. Immunol*. 157:4741–4745.
12. Carretero, M., C. Cantoni, T. Bellon, C. Bottino, R. Biassoni, A. Rodriguez, J.J. Perezvillar, L. Moretta, A. Moretta, and M. López-Botet. 1997. The CD94 and NKG2-A C-type lectins covalently assemble to form a natural killer cell inhibitory receptor for HLA class I molecules. *Eur. J. Immunol*. 27: 563–567.
13. Colonna, M., and J. Samaridis. 1995. Cloning of immunoglobulin-superfamily members associated with HLA-C and HLA-B recognition by human natural killer cells. *Science (Wash. DC)*. 268:367–368.
14. Wagtmann, N., R. Biassoni, C. Cantoni, S. Verdiani, M.S. Malnati, M. Vitale, C. Bottino, L. Moretta, A. Moretta, and E.O. Long. 1995. Molecular clones of the p58 NK cell receptor reveal immunoglobulin-related molecules with diversity in both the extra- and intracellular domains. *Immunity*. 2: 439–449.
15. Rojo, S., D.N. Burshtyn, E.O. Long, and N. Wagtmann. 1997. Type I transmembrane receptor with inhibitory function in mouse mast cells and NK cells. *J. Immunol*. 158:9–12.
16. Wang, L.L., I.K. Mehta, P.A. Leblanc, and W.M. Yokoyama. 1997. Cutting edge: mouse natural killer cells express GP49b1, a structural homologue of human killer inhibitory receptors. *J. Immunol*. 158:13–17.
17. Nakamura, M.C., E.C. Niemi, M.J. Fisher, L.D. Shultz, W.E. Seaman, and J.C. Ryan. 1997. Mouse Ly-49A interrupts early signaling events in natural killer cell cytotoxicity and functionally associates with the SHP-1 tyrosine phosphatase. *J. Exp. Med*. 185:673–684.
18. 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*. 5:629–638.
19. Wong, S., J.D. Freeman, C. Kelleher, D. Mager, and F. Takei. 1991. Ly-49 multigene family. New members of a superfamily of type II membrane proteins with lectin-like domains. *J. Immunol*. 147:1417–1423.
20. Smith, H.R.C., F.M. Karlhofer, and W.M. Yokoyama. 1994. Ly-49 multigene family expressed by IL-2–activated NK cells. *J. Immunol*. 153:1068–1079.
21. Colonna, M. 1997. Specificity and function of immunoglobulin superfamily NK cell inhibitory and stimulatory receptors. *Immunol. Rev*. 155:127–133.
22. Lanier, L.L., B. Corliss, and J.H. Phillips. 1997. Arousal and inhibition of human NK cells. *Immunol. Rev*. 155:145–154.
23. Long, E.O., D.N. Burshtyn, W.P. Clark, M. Peruzzi, S. Rajagopalan, S. Rojo, N. Wagtmann, and C.C. Winter. 1997. Killer cell inhibitory receptors: diversity, specificity, and function. *Immunol. Rev*. 155:135–144.
24. Moretta, A., R. Biassoni, C. Bottino, D. Pende, M. Vitale, A. Poggi, M.C. Mingari, and L. Moretta. 1997. Major histocompatibility complex class I–specific receptors on human natural killer and T lymphocytes. *Immunol. Rev*. 155:105–117.
25. Reyburn, H., O. Mandelboim, M. Valesgomez, E.G. Sheu, L. Pazmany, D.M. Davis, and J.L. Strominger. 1997. Human NK cells: their ligands, receptors and functions. *Immunol. Rev*. 155:119–125.
26. Salter, R.D., H.W. Chan, R. Tadikamalla, and D.A. Lawlor. 1997. Domain organization and sequence relationship of killer cell inhibitory receptors. *Immunol. Rev*. 155:175–182.
27. Selvakumar, A., U. Steffens, and B. Dupont. 1997. Polymorphism and domain variability of human killer cell inhibitory receptors. *Immunol. Rev*. 155:183–196.
28. Valiante, N.M., K. Lienert, H.G. Shilling, B.J. Smits, and P. Parham. 1997. Killer cell receptors: keeping pace with MHC class I evolution. *Immunol. Rev*. 155:155–164.
29. Bottino, C., S. Sivori, M. Vitale, C. Cantoni, M. Falco, D. Pende, L. Morelli, R. Augugliaro, G. Semenzato, R. Biassoni, et al. 1996. A novel surface molecule homologous to the p58/p50 family of receptors is selectively expressed on a subset of human natural killer cells and induces both triggering of cell functions and proliferation. *Eur. J. Immunol*. 26: 1816–1824.
30. Mandelboim, O., D.M. Davis, H.T. Reyburn, M. Valesgomez, E.G. Sheu, L. Pazmany, and J.L. Strominger. 1996. Enhancement of class II–restricted T cell responses by costimulatory NK receptors for class I MHC proteins. *Science (Wash. DC)*. 274:2097–2100.
31. Mason, L.H., S.K. Anderson, W.M. Yokoyama, H.R.C. Smith, R. Winklerpickett, and J.R. Ortaldo. 1996. The Ly-49D receptor activates murine natural killer cells. *J. Exp. Med*. 184:2119–2128.
32. Cambier, J.C. 1995. New nomenclature for the Reth motif (or ARH1/TAM/ARAM/YXXL). *Immunol. Today*. 16:110.
33. Weiss, A., and D.R. Littman. 1994. Signal transduction by lymphocyte antigen receptors. *Cell*. 76:263–274.
34. Olcese, L., A. Cambiaggi, G. Semenzato, C. Bottino, A. Moretta, and E. Vivier. 1997. Human killer cell activatory receptors for MHC class I molecules are included in a multimeric complex expressed by natural killer cells. *J. Immunol*. 158:5083–5086.
35. Ono, M., S. Bolland, P. Tempst, and J.V. Ravetch. 1996. Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor FcγRIIB. *Nature (Lond.)*.

383:263–266.
36. D'Ambrosio, D., K.L. Hippen, S.A. Minskoff, I. Mellman, G. Pani, K.A. Siminovitch, and J.C. Cambier. 1995. Recruitment and activation of PTP1C in negative regulation of antigen receptor signaling by FcγRIIb1. *Science (Wash. DC)*. 268: 293–297.
37. Nadler, M.J.S., B.B. Chen, J.S. Anderson, H.H. Wortis, and B.G. Neel. 1997. Protein-tyrosine phosphatase SHP-1 is dispensable for FcγRIIB-mediated inhibition of B cell antigen receptor activation. *J. Biol. Chem*. 272:20038–20043.
38. Burshtyn, D.N., W. Yang, T. Yi, and E.O. Long. 1997. A novel phosphotyrosine motif with a critical amino acid at position −2 for the SH2 domain-mediated activation of the tyrosine phosphatase SHP-1. *J. Biol. Chem*. 272:13066–13072.
39. Ono, M., H. Okada, S. Bolland, S. Yanagi, T. Kurosaki, and J.V. Ravetch. 1997. Deletion of SHIP or SHP-1 reveals two distinct pathways for inhibitory signaling. *Cell*. 90:293–301.
40. Gupta, N., A.M. Scharenberg, D.N. Burshtyn, N. Wagtmann, M.N. Lioubin, L.R. Rohrschneider, J.P. Kinet, and E.O. Long. 1997. Negative signaling pathways of the killer cell inhibitory receptor and FcγRIIb1 require distinct phosphatases. *J. Exp. Med*. 186:473–478.
41. Nada, S., M. Okada, A. MacAuley, J.A. Cooper, and H. Nakagawa. 1991. Cloning of a complementary DNA for a protein-tyrosine kinase that specifically phosphorylates a negative regulatory site of p60c-src. *Nature (Lond.)*. 351:69–72.
42. Vuica, M., S. Desiderio, and J.P. Schneck. 1997. Differential effects of B cell receptor and B cell receptor–FcγRIIb1 engagement on docking of Csk to GTPase-activating protein (GAP)–associated p62. *J. Exp. Med*. 186:259–267.
43. Olcese, L., P. Lang, F. Vely, A. Cambiaggi, D. Marguet, M. Blery, K.L. Hippen, R. Biassoni, A. Moretta, L. Moretta, et al. 1996. Human and mouse killer-cell inhibitory receptors recruit PTP1C and PTP1D protein tyrosine phosphatases. *J. Immunol*. 156:4531–4534.
44. Kharitonenkov, A., Z. Chen, I. Sures, H. Wang, J. Schilling, and A. Ullrich. 1997. A family of proteins that inhibit signalling through tyrosine kinase receptors. *Nature (Lond.)*. 386: 181–186.
45. Brown, M.G., A.A. Scalzo, K. Matsumoto, and W.M. Yokoyama. 1997. The natural killer gene complex: a genetic basis for understanding natural killer cell function and innate immunity. *Immunol. Rev*. 155:53–65.
46. Wagtmann, N., S. Rojo, E. Eichler, H. Mohrenweiser, and E.O. Long. 1997. A new human gene complex encoding the killer cell inhibitory receptors and related monocyte/macrophage receptors. *Curr. Biol*. 7:615–618.
47. Arm, J.P., M.F. Gurish, D.S. Reynolds, H.C. Scott, C.S. Gartner, K.F. Austen, and H.R. Katz. 1991. Molecular cloning of gp49, a cell-surface antigen that is preferentially expressed by mouse mast cell progenitors and is a new member of the immunoglobulin superfamily. *J. Biol. Chem*. 266: 15966–15973.
48. Samaridis, J., and M. Colonna. 1997. Cloning of novel immunoglobulin superfamily receptors expressed on human myeloid and lymphoid cells: structural evidence for new stimulatory and inhibitory pathways. *Eur. J. Immunol*. 27:660–665.
49. Cella, M., C. Dohring, J. Samaridis, M. Dessing, M. Brockhaus, A. Lanzavecchia, and M. Colonna. 1997. A novel inhibitory receptor (ILT3) expressed on monocytes, macrophages, and dendritic cells involved in antigen processing. *J. Exp. Med*. 185:1743–1751.
50. Cosman, D., N. Fanger, L. Borges, M. Kubin, W. Chin, L. Peterson, and M.-L. Hsu. 1997. A novel immunoglobulin superfamily receptor for cellular and viral MHC class I molecules. *Immunity*. 7:273–282.
51. Meyaard, L., G.J. Adema, C. Chang, E. Woollatt, G.R. Sutherland, L.L. Lanier, and J.H. Phillips. 1997. LAIR-1, a novel inhibitory receptor expressed on human mononuclear leukocytes. *Immunity*. 7:283–290.
52. Kubagawa, H., P.D. Burrows, and M.D. Cooper. 1997. A novel pair of immunoglobulin-like receptors expressed by B cells and myeloid cells. *Proc. Natl. Acad. Sci. USA*. 94:5261–5266.
53. Hayami, K., D. Fukuta, Y. Nishikawa, Y. Yamashita, M. Inui, Y. Ohyama, M. Hikida, H. Ohmori, and T. Takai. 1997. Molecular cloning of a novel murine cell-surface glycoprotein homologous to killer cell inhibitory receptors. *J. Biol. Chem*. 272:7320–7327.
54. Katz, H.R., E. Vivier, M.C. Castells, M.J. McCormick, J.M. Chambers, and K.F. Austen. 1996. Mouse mast cell gp49B1 contains two immunoreceptor tyrosine-based inhibition motifs and suppresses mast cell activation when coligated with the high-affinity Fc receptor for IgE. *Proc. Natl. Acad. Sci. USA*. 93:10809–10814.
55. Reyburn, H.T., O. Mandelboim, M. Vales-Gomez, D.M. Davis, L. Pazmany, and J.L. Strominger. 1997. The class I MHC homologue of human cytomegalovirus inhibits attack by natural killer cells [see comments]. *Nature (Lond.)*. 386: 514–517.
56. Fan, Q.R., L. Mosyak, C.C. Winter, N. Wagtmann, E.O. Long, and D.C. Wiley. 1997. The human natural killer cell inhibitory receptor structure resembles hematopoietic receptors. *Nature (Lond.)*. 389:96–100.
57. 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*. 158:4026–4028.
58. Mandelboim, O., H.T. Reyburn, E.G. Sheu, M. Vales-Gomez, D.M. Davis, L. Pazmany, and J.L. Strominger. 1997. The binding site of NK receptors on HLA-C molecules. *Immunity*. 6:341–350.
59. Colonna, M., E.G. Brooks, M. Falco, G.B. Ferrara, and J.L. Strominger. 1993. Generation of allospecific natural killer cells by stimulation across a polymorphism of HLA-C. *Science (Wash. DC)*. 260:1121–1124.
60. Gumperz, J.E., V. Litwin, J.H. Phillips, L.L. Lanier, and P. Parham. 1995. The Bw4 public epitope of HLA-B molecules confers reactivity with natural killer cell clones that express NKB1, a putative HLA receptor. *J. Exp. Med*. 181:1133–1144.