authors have in fact already isolated two different members of the family. The availability of the entire genome sequence should permit the resolution of this issue in the very near future.

The new receptor's dual specificity for IgM and IgA was not a predictable feature for an IgM receptor on B cells and monocytes. Although the polyspecificity of the pIgR transporter is easy to rationalize, it is more difficult to articulate the type(s) of selective advantage that could be conferred by this dual specificity in the context of B cells, monocytes and macrophages. This is especially true in view of the fact that there is another IgA receptor (CD89) that does not bind IgM. This receptor does not belong to the chromosome 1 family although it also contains two immunoglobulin-related domains. Its gene is found on human chromosome 19 (**Fig. 1**)[1]. No mouse counterpart has been isolated so far. CD89 is expressed on, among other cells, monocytes where the new Fcα/μR is also expressed. Therefore human monocytes express at least two IgA receptors and their respective functions will need to be dissected.

Another unexpected feature of the Fcα/μR relates to the structure of its binding site. One of the hallmarks of the FcR family is that these receptors resemble their own ligands: they contain immunoglobulin-related domains capable of binding the immunoglobulin ligand. Until now, all Fc receptors in this family have been found to contain at least two of these immunoglobulin-related domains. The recent solving of the crystal structure of two different FcR-immunoglobulin complexes has shown that the FcR-immunoglobulin binding interactions require more than one of the immunoglobulin-related domains of the FcR[7,8]. It is therefore remarkable that the new Fcα/μR contains only one immunoglobulin-related domain and is still capable of binding both IgM and IgA with high affinity. The authors point to the clear homology between the single immunoglobulin domain of the FcRα/μR and the first of the five immunoglobulin-related domains of the pIgR. It is known that the latter domain is required for IgM and IgA binding, but it is thought that the main common component of dimeric IgA and pentameric IgM is binding the J chain[9]. On that basis, one would expect that the single immunoglobulin-related domain of the FcRα/μR would also bind the J chain, but this does not appear to be the case. Monomeric IgA, which does not have J chain, also binds to Fcα/μR. Therefore, one can predict that a new type of intermolecular binding must exist between IgM or IgA and the single immunoglobulin domain of the Fcα/μR.

Whether a single member or the first-born in a new family, Fcα/μR (and the potential siblings) are likely to play a fundamental role in mediating IgM and IgA effector functions in both innate and adaptive immunity and may in fact be the missing link between the two. The genetic targeting of this receptor will help to determine its precise role regarding these functions and to define its full functional impact in the immune defense system.

1. Daeron, M. *Annu. Rev. Immunol.* **15**, 203–234 (1997).
2. Shibuya, A. *et al. Nature Immunol.* **1**, 441–447 (2000).
3. Ehrenstein, M. R., O'Keefe, T. L., Davies, S. L. & Neuberger, M. S. *Proc. Natl Acad. Sci. USA* **95**, 10089–10093 (1998).
4. Boes, M. *et al. Proc. Natl Acad. Sci. USA* **97**, 1184–1189 (2000).
5. Ohno, T., Kubagawa, H., Sanders, S. K. & Cooper, M. D. *J. Exp. Med.* **172**, 1165–1175 (1990).
6. Nakamura, T., Kubagawa, H., Ohno, T. & Cooper, M. D. *J. Immunol.* **151**, 6933–6941 (1993).
7. Sondermann, P., Huber, R., Oosthuizen, V. & Jacob, U. *Nature* **406**, 267–273 (2000).
8. Garman, S. C., Wurzburg, B. A., Tarchevskaya, S. S., Kinet, J. P. & Jardetzky, T. S. *Nature* **406**, 259–266 (2000).
9. Hendrickson, B. A. *et al. J. Exp. Med.* **182**, 1905–1911 (1995).

Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts 02215, USA.
(jkinet@caregroup.harvard.edu)

Signaling for cytotoxicity

Bice Perussia

Once natural killer cells identify their targets they engage their lysis machinery. Spontaneous, unlike antibody-dependent, cytotoxicity predominantly uses a Ras-independent pathway to accomplish this activation.

One mechanism by which sensitive target cells are lysed by natural killer (NK) cells and cytotoxic T lymphocytes (CTLs) is through the release of cytotoxic mediators (perforin and granzymes). These are stored in cytoplasmic granules preformed in NK cells and generated in CTLs upon peptide–major histocompatibility complex (MHC) recognition by the T cell receptor (TCR)-CD3 complex and consequent functional maturation to effector cells. The interaction between cellular ligands on the target cell and specific receptors on the effector lymphocytes leads to aggregation of the receptors. This initiates a series of biochemical events responsible for the redistribution of the granules and their concentrated constituents to the area of cell-cell contact, where they are released to execute the lysis of target cells. In this issue of *Nature Immunology* Jiang *et al.* elucidate this pathway almost from start to finish[1].

NK cells lyse target cells *via* spontaneous or antibody-dependent cytotoxicity (ADCC). In both cases, phosphorylation resulting from engagement of receptors involved in target cell recognition results in the activation of specific protein kinases (the primary kinases being Src family kinases and Syk[2]) that are central to NK cell functions, including granule release. Also, in both cases, pharmacological inhibition of members of the mitogen-activated protein kinase (MAPK) family, specifically extracellular signal-regulated kinase (ERK) and p38, or overexpression of nonfunctional, mutated forms of these kinases, inhibits granule redistribution and release after target cell binding. Several intermediate molecules in the pathway of MAPK activation have been identified, but the exact sequence in which these kinases influence each other's activity had not been established. In this issue, Jiang *et al*. propose that the redistribution of lytic granules (as determined by the movement of perforin and granzymes) in primary NK cells during spontaneous cytotoxicity depends on the receptor-triggered sequential recruitment and activation of a Rac1→p21–activated kinase 1 (PAK1)→MAPK kinase (MEK)→ERK pathway that is activated in a Ras-independent

fashion by phosphatidylinositol-3 kinase (PI3K). This sequence of signal transducers functions in a unique pathway in NK cells and through activation of the MAPK ERK signaling element leads to tumor cell lysis.

In ADCC, the most relevant receptor signaling for ERK-dependent granule release is FcγRIIIA, which recognizes IgG-coated target cells. In spontaneous cytotoxicity, however, several distinct surface proteins are likely involved. Only a few of these "activating" receptors have been identified, including the β_2 integrin family member CD18-CD11b (also known as Mac-1 or CR3) and NKG2D. Whether granule redistribution in all cases depends on activation of a single predominant pathway, or represents the net result of the activation of and crosstalk between several possibly different ones specifically elicited by distinct receptors, remains to be established. By examining the pathway proposed by Jiang *et al.* one can begin to establish common features and differences between this and previously reported pathways, such as the FcγRIIIA or β integrins which are triggered upon engagement of defined receptors and which participate in granule redistribution and/or release in ADCC or spontaneous cytotoxicity (**Fig. 1**).

Upon FcγRIIIA ligation, the GTP-GDP exchange protein encoded by the *Ras* protooncogene is also activated, as supported by the transient accumulation of the GTP-bound form of Ras[3]. The p52 and the p46 forms of the oncoprotein Shc become phosphorylated and Shc immunoprecipitates from FcγRIIIA-stimulated NK cells containing Grb2, a Src homology 2 (SH2) and SH3 domain–containing protein, and an undefined 145-kD phosphoprotein. Grb2 not only binds to Shc, but its SH2 domain binds to the p36 LAT tyrosine phosphoprotein. Although a direct link between Ras activation and formation of these complexes is not established, the same types of complexes participate to the activation of Ras.

The involvement of Ras in FcγIIIRA activation is further supported by the transient tyrosine phosphorylation of the protooncogene Vav, which associates with Syk[4]. Additional complexes identified are those formed by Cbl in association with Grb2. Interestingly, the Shc-Grb2 complexes seem to associate preferentially, if not exclusively, to the FcγRIIIA associated with the ζ chain, but not the γ chain. This suggests the possibility that the two types of receptor may couple to distinct biochemical pathways, the role of which in granule, exocytosis, remains to be established. In both cases, however, PI3K is detected associated to the receptor, and its enzymatic activity is necessary for granule release to occur[2,4,5]. Thus, a Ras-dependent pathway, unlike the Rac-1 dependent one discussed by Jiang *et al.* for spontaneous cytotoxicity, seems to correlate with ERK activation in ADCC.

With the exception of the most proximal events (involving the Src family, ZAP-70 and Syk kinases in the case of FcγRIIIA, and the proline-rich Pyk2 tyrosine kinase in the case of β_2 integrins[6]) the same type of complexes and kinases discussed for FcγRIIIA-mediated signaling are detected in β_1 integrin–stimulated NK cells[7]. In addition, analogous to what is reported by Jiang *et al.*, β_1 integrin ligation is accompanied by the activation of the small G protein Rac1 and the Rac guanine nucleotide-exchange factor p95 Vav, both of which control p38 MAPK activation in this case[6]. Interestingly, NK cells overexpressing Vav, but not a mutant without exchange-factor activity, mediate significantly increased ADCC and spontaneous cytotoxicity. Conversely, expression of a dominant-negative Rac-1 correlates with decreased NK–target cell conjugate formation and decreased polarization of lytic

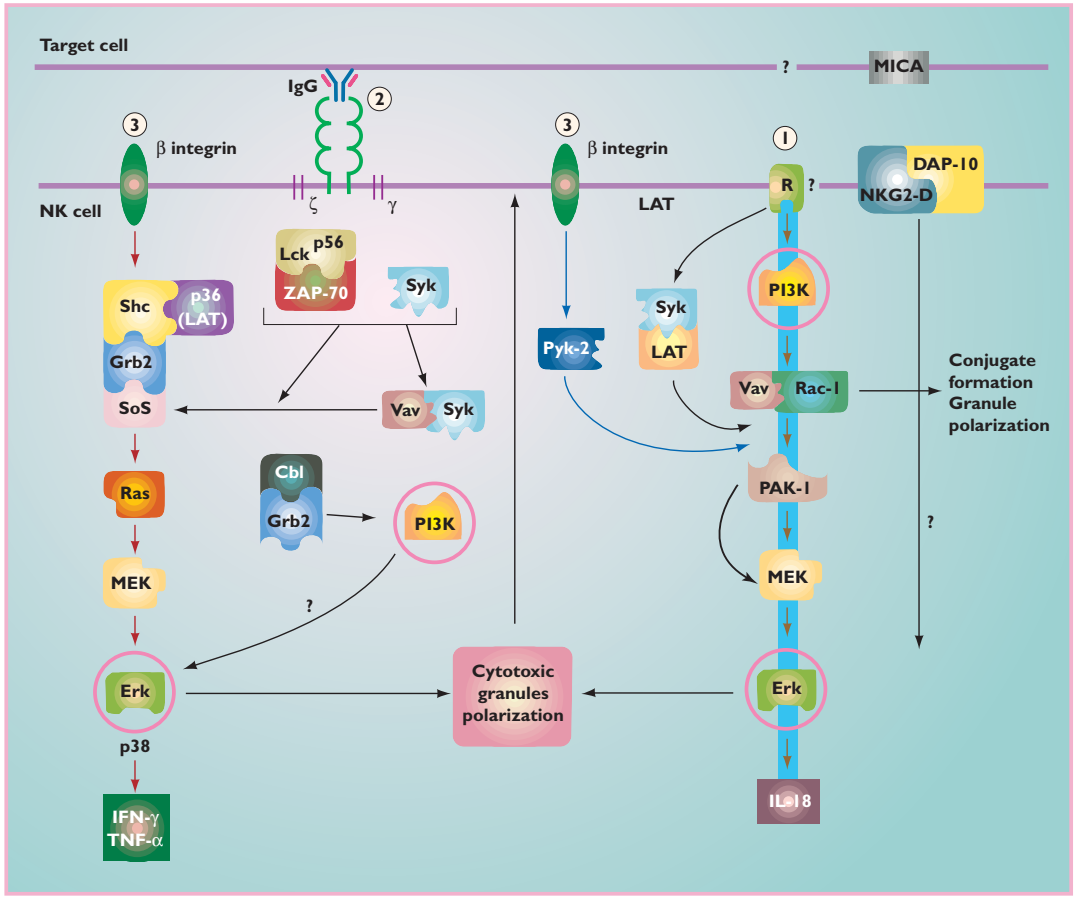

Figure 1. Signaling events in NK cell-mediated cytotoxicity. The molecules (receptors, adapters, and kinases) reported to be involved in signaling pathways activated in NK cells (bottom) upon target cell recognition (top) are schematically indicated. Left: signaling during ADCC, initiated upon engagement of FcγRIIIA by IgG–target cell immune complexes. Right: signaling during spontaneous cytotoxicity, initiated upon engagement of undefined (R?) or defined (NKG2D) "activating" receptors with their undefined (?) or known (MICA) ligands, respectively. The pathways involved in the signaling initiated upon engagement of β integrins by their target adhesion molecules are indicated on both sides (arrow pointing toward the effector-target cell contact area). Kinases central to cytotoxic granules polarization are circled; effector functions known to depend on the activity of specific intermediates are boxed; ?, indicates steps to be still completely defined.

granules to the area of contact between killer and target in those NK cells still capable of conjugate formation[8]. In spontaneous cytotoxicity, like during ADCC, LAT is phosphorylated, and the overexpression of LAT results in enhancement of both ADCC and spontaneous cytotoxicity. Together, these data pinpoint similarities between the pathways induced during ADCC and spontaneous cytotoxicity. It will be interesting to determine whether the events leading to ERK activation in the FcγRIIIA-mediated granule release are the same as defined by Jiang *et al*.

The pathway defined by Jiang *et al*. in NK cells seems to differ from the classical TCR signaling pathway triggered by ligation of peptide-MHC. That pathway involves association of Grb2-Sos complexes with the TCR and recruitment of Ras, rather than Rac, to the plasma membrane, with consequent Raf-1 binding to GTP-bound Ras, Raf-1 activation, phosphorylation of the dual MEK-1 kinase and ERK phosphorylation and activation. Two recent reports, however, have presented data that support similarities with NK cells in the TCR-induced pathways responsible for cytoskeletal reorganization in T cells. A Vav-dependent Rac-1 phosphorylation event may be induced upon interaction of the TCR with peptide-MHC on antigen presenting cells (APC). This leads to the cytoskeletal reorganization that allows antigen-presenting cell–T cell conjugate formation and the instigation of the "immunological synapse"[9]. In addition, Rac-1 was found to act both downstream and upstream of PI3K in the pathway leading to cytoskeleton rearrangements upon TCR engagement[10].

The results of Jiang *et al.* show that ERK activation, and the pathways leading to it, are abrogated in PI3K–inhibited NK cells. All of the pathways can be restored in these cells, except in MAPK-inhibited cells, upon overexpression of wild-type, but not negative-mutant Rac-1 or PAK-1, which convincingly supports the conclusion that PI3K plays an essential role in regulation of the MAPK pathway leading to granule redistribution in spontaneous cytotoxicity. The lack of effect of pharmacological inhibitors of PI3K on spontaneous cytotoxicity mediated by cloned human NK cells[2] had previously excluded a role for this lipid kinase in spontaneous cytotoxicity. We can speculate and attempt to reconcile Jiang's data with the previous inhibitor studies, taking into consideration several points. The first is that conclusions drawn from experiments performed with pharmacological inhibitors suffer from caveats related to possible unrecognized effects on proteins that are different to those for which they are known to be specific. On the other hand, overexpression of genes encoding specific kinases or adapter proteins may significantly alter the physiological balance of the different factors involved in specific cellular functions. Extreme situations can be created under these conditions. However, with the combined use of several approaches and relevant controls, one can reasonably assume that the data obtained under these experimental conditions reflects actual possibilities. These may not equate exactly with the reality, but likely are its best approximation.

Second, cytotoxicity can be measured only by allowing effector-target cell contact. Numerous molecules on the effector cells, including adhesion molecules, are likely to bind their own ligands on the target cells at the same time that receptors recognizing specific "activating" ligands are engaged. Depending on the relative numbers and affinities of the different interactions involved, several biochemical pathways may be elicited, and their contribution to the observed net result cannot be dissected independently. For example, signals are likely to be transduced, during both ADCC and spontaneous cytotoxicity, by ICAM-1 binding to its ligand LFA-1 (another β_2 integrin family member) the neutralization of which results in inhibition of both ADCC and spontaneous cytotoxicity. Expression of adhesion molecules on target cells or on the clonal NK cell populations used in different laboratories may vary and so, therefore, may the number and/or interactions of the pathways generated. Polyclonal cell populations best approximate physiological conditions.

Based on these considerations, it seems reasonable to accept that the results of Jiang *et al.* at best recapitulate what happens in NK cells. However, the possibility remains that other PI3K-independent pathways, possibly with a minor role in granule release, are elicited during spontaneous cytotoxicity. In this regard, the data of Jiang *et al.* actually suggest the possible existence of a Rac-1 independent (likely PI3K-independent) pathway induced upon NK-target cell interaction. Whereas the levels of active MAPK in the MEK inhibitor–treated NK92 cell line expressing active Rac1 appear similar to those in the DMSO-treated control cells infected with the same viral construct, in primary NK cells they remain lower. The question of whether such a pathway exists awaits additional work. The role in granule release of a possible minor PI3K– and Rac-1–independent pathway could be addressed by testing whether a nonoptimal restoration of the cytotoxic potential of the MEK inhibitor–treated NK cells expressing active Rac-1 is observed in cytotoxicity assays with serially lower numbers of effector cells.

Finally, in a broader context, the data of Jiang *et al.* may have relevance to the mechanisms underlying functions not only of NK cells but also of other effectors of innate immunity. "Activating" immune receptors are being identified on most leukocyte types. They share accessory signal-transducing subunits that are associated with distinct ligand binding subunits. Within the cytoplasmic domains of one of these, DAP-10 (associated with the NKG2D receptor for stress-induced MICA on NK and T cells), an SH2 domain-binding site capable of recruiting PI3K has been identified[11]. Additional transmembrane adapter proteins, like KAP10, that can directly bind PI3K and Grb2 are being cloned from the effector cells in innate immunity[12]. Based on the data by Jiang *et al*., it may be possible that pathways similar to that reported here for granule redistribution in spontaneous cytotoxicity are induced by these receptors. It will be important to determine whether PI3K is central to the signal transduction events elicited upon stimulation of these receptors, whether it plays a common role or one more specific to the functions of the cells expressing them, and to exploit the knowledge derived from this report to identify new activating receptors.

1. Jiang, K. *et al. Nature Immunol.* **1**, 419–425 (2000).
2. Brumbaugh, K. M. *et al. J. Exp. Med.* **186**, 1965–1974 (1997).
3. Perussia, B. *Curr. Top. Microbiol. Immunol.* **230**, 63–88 (1998).
4. Galandrini, R., Palmieri, G., Piccoli, M., Frati, L. & Santoni, A. *J. Immunol.* **162**, 3148–3152 (1999).
5. Bonnema, J. D., Karnitz, L. M., Schoon, R. A., Abraham, R. T. & Leibson, P. J. *Ann. NY Acad. Sci.* **766**, 209–213 (1994).
6. Gismondi, A. *et al. J. Immunol.* **164**, 2272–2276 (2000).
7. Mainiero, F. *et al. Immunity* **12**, 7–16 (2000).
8. Billadeau, D. *et al. J. Exp. Med.* **188**, 549–559 (1998).
9. Huang J., Tilly, D., Altman, A., Sugie, K., & Grey, H. M. *Proc. Natl Acad. Sci. USA* **97**, 10923–10929 (2000).
10. Genot, E. M., Arrieumerlou, C., Ku, G., Burgering, B. M., Weiss, A. & Krammer, I. *Mol. Cell. Biol.* **20**, 5469–5478 (2000).
11. Wu, J. *Science* **285**, 730–732 (1999).
12. Chang, C. *et al. J. Immunol.* **163**, 4651–4654 (1999).

Kimmel Cancer Center, Jefferson Medical College, Department of Microbiology and Immunology, BLSB 750, 233 S. 10th Street, Philadelphia, PA 19107, USA. (bperussia@hendrix.jci.tju.edu)