

The absence of Grb2-associated binder 2 (Gab2) does not disrupt NK cell development and functions

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Abstract: Scaffolding molecules bind simultaneously and link together various components of signal-transduction pathways. Grb2-associated binder 2 (Gab2) is a scaffolding protein required for FcγR-initiated allergic responses in mast cells and FcγR-mediated phagocytosis in macrophages, where it links IgE and IgG receptors to the phosphatidylinositol-3 kinase (PI-3K) pathway. The FcγR expressed by natural killer (NK) cells triggers antibody-dependent cellular cytotoxicity (ADCC). We show here that mouse NK cells express Gab2 and that although PI-3K was required for ADCC, this FcγR-mediated function was normal in *Gab2*^{-/-} NK cells. Moreover, NK cell development, spontaneous cytotoxicity, and responses to and production of cytokines were not perturbed in *Gab2*^{-/-} mice. Considering the striking differences between the signaling requirements of FcγR in macrophages and NK cells, our findings suggest that the organization of signal transduction downstream of the same FcR can be cell type-specific. Conversely, Gab family members Gab1, Gab2, and Gab3 may play specific roles in different leukocytes. As pharmacological targeting of Gab2 in mast cells is a potential strategy to treat allergy, our results suggest prudence, as NK cells may participate in IgE-mediated anaphylaxis in a Gab2-independent manner. *J. Leukoc. Biol.* 76: 896–903; 2004.

Key Words: cell-mediated cytotoxicity · intracellular signaling · FcR functions

INTRODUCTION

Natural killer (NK) cells are a subset of lymphocytes able to kill tumor cells and infected cells without prior stimulation. NK cells also produce proinflammatory and antimicrobial cytokines and chemokines that modulate immune responses [1]. Although we appreciate their effector functions in innate immunity and their regulatory functions in shaping adaptive immunity, the intracellular signals that regulate NK cells functions are not fully understood [2]. NK cells express, at their surface, multiple molecules capable of triggering cytotoxicity, among which is a low-affinity Fc receptor for immunoglobulin

G (IgG; FcγR)IIIa, also known as CD16, which is responsible for antibody (Ab)-dependent cellular cytotoxicity (ADCC) [3, 4]. In addition to ADCC, ligation of CD16 on NK cells induces cytokine release, notably interferon-γ (IFN-γ) and tumor necrosis factor α.

Like all multichain immune-recognition receptors, CD16 lacks intrinsic enzymatic activity. The association of these receptors with adaptor signaling subunits that contain immune receptor-based tyrosine-activating motifs (ITAMs) allows signal transduction. Human CD16 can couple with homodimers or heterodimers composed of the signaling chains CD3ζ or Fc receptor for IgE (FcεR)Iγ, whereas mouse CD16 can only bind to FcεRIγ. Ligation of CD16 by Ab-antigen (Ag)-immune complexes or by specific monoclonal Ab (mAb) activates membrane-associated Src tyrosine kinases, which phosphorylate tyrosine residues on the ITAMs. Syk family tyrosine kinases Syk and ζ-associated protein-70 (ZAP-70) are then recruited to the phosphorylated ITAMs and become activated. Syk tyrosine kinases are essential for signal transduction through CD16 and other ITAM-bearing receptors in NK cells [2]. Phosphatidylinositol-3 kinase (PI-3K) enzymes are also activated by CD16 and have been shown to participate in granule exocytosis and ADCC activity in human NK cells [5, 6]. It is clear that the Syk and PI-3K pathways may be crucial in different forms of NK cell-mediated cytotoxicity [7–10]; however, it is not known if and how they are linked together.

A potential molecular link between Syk and PI-3K in NK cells is Grb2-associated binder 2 (Gab2), a member of the daughter of sevenless (Dos)/Gab family of scaffolding proteins, which includes mammals Gab1, Gab2, and Gab3, *Drosophila* homologue Dos, and *Caenorhabditis elegans* homologue suppressor of clear 1. These adaptor molecules are devoid of any catalytic function but contain several binding sites for signaling molecules such as Grb2, Shc, Src homology-2-containing tyrosine phosphatase, and the p85 subunit of the PI-3K as well as a highly conserved pleckstrin homology domain, which mediates recruitment to the plasma membrane through interaction with phospholipids. In vitro studies have shown the implication of Gab2 in growth factor, cytokine, and multichain immune-recognition receptor signaling [11–13].

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Although lymphoid and hematopoietic development was normal in *Gab2*^{-/-} mice, mast cell responses to in vitro and in vivo stimulation of the high-affinity FcεRI were defective [14]. Thus, allergic reactions, such as anaphylaxis or cutaneous reactions, were markedly impaired. *Gab2* was shown to play a key role in PI-3K activation in downstream FcεRI, suggesting that at least in mast cells, the ITAM-bearing and Syk-dependent FcεRI is linked to the PI-3K pathway by *Gab2* [14].

A similar organization has been described in macrophages where *Gab2* plays a key role in downstream PI-3K-dependent FcR signaling [15]. FcR triggering in macrophages initiates phagocytosis. Mouse macrophages express three receptors for IgGs: the high-affinity receptor FcγRI (CD64), capable of binding monomeric IgG, and the low-affinity FcγRIII (CD16), which only binds multimeric IgGs. The third FcR expressed by mouse macrophages is the inhibitory FcγRIIB (CD32), also a low-affinity receptor (reviewed in ref. [16]). FcγR-mediated phagocytosis was defective in *Gab2*^{-/-} bone marrow-derived macrophages (BMM): This correlated with a decreased activation of the PI-3K pathway in *Gab2*^{-/-} macrophages, as attested by impaired phosphorylation of Akt, a downstream target of PI-3K [15].

Thus, *Gab2* is a key component of FcR signal transduction in mast cells and in macrophages. In these two cell types, *Gab2* links together multichain immune-recognition receptors and the PI-3K pathway. We hypothesized that *Gab2* could be an important player in NK cell-mediated ADCC in downstream CD16 signaling and could also participate in several aspects of NK cell biology. First, macrophages and NK cells share some of the crucial molecules involved in FcR signal transduction. For example, Src, Syk, and PI-3K are key components of the activation cascades that trigger FcγR-mediated phagocytosis in macrophages and FcγRIII-mediated ADCC in NK cells [5, 6, 17, 18]. In addition to its potential role in signal transduction downstream of FcγR in NK cells, the various *Gab* family members have been associated with other receptors that mediate functions relevant to NK cell biology. In particular, *Gab2* participates in signal transduction downstream of c-kit and fetal liver tyrosine kinase 3 ligand (Flt3; reviewed in ref. [19]), two receptor tyrosine kinases involved in NK cell development [20]. Moreover, *Gab2* participates in the signal transduction of the receptors for interleukin (IL)-2 and IL-15, two crucial cytokines for NK cell expansion in vitro and NK cell development in vivo, respectively [20].

With these premises, we analyzed the expression pattern of *Gab* family members in NK cells and studied NK cell development and effector functions in *Gab2*^{-/-} mice. We found that NK cells expressed *Gab2* protein, albeit at lower levels compared with mast cells and macrophages. Like mast cells and in contrast to macrophages, NK cells did not express transcripts for *Gab1*. *Gab2* and *Gab3* transcripts were present in NK cells. *Gab2*^{-/-} NK cells developed with no apparent phenotype, responded to CD16 triggering by mediating normal ADCC and by producing and secreting IFN-γ, were activated by and proliferated in response to IL-2 in vitro, and could kill tumor cells by spontaneous cytotoxicity. These results are compatible with a redundant role of *Gab2* in NK cells and with a cell-specific organization of FcR signal-transduction pathways.

MATERIALS AND METHODS

Mice

Gab2^{-/-} mice were described previously [14]. We crossed the original *Gab2*^{-/-} mice (129j×C57BL/6 background) for six generations onto the C57BL/6 background. C57BL/6, C57BL/6.*Gab2*^{-/-}, and C57BL/6.*Rag2*^{-/-} mice were kept at the Central Animal Facilities of The Pasteur Institut (Paris, France) and were used for experiments at 6–12 weeks of age. All protocols for animal experiments were reviewed by the Central Animal Facilities of The Pasteur Institut and were done in accordance with guidelines approved by the French Ministry of Agriculture.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA of various cells or spleen was extracted using the RNable kit (Eurobio). RT-PCR was performed using specific oligonucleotides. Primers for *Gab1* and *Gab3* have been published previously [21]. We designed primers for *Gab2* (*Gab2* forward, 5'-CAACCTGAACTTCTGTGAGC-3'; *Gab2* reverse, 5'-TGCTGGACAAGGTAGGCTGC-3'), which do not recognize *Gab1* and *Gab3* cDNA sequences found in the database. Standard PCR conditions were used to amplify *Gab2* for 38 cycles and *Gab 1* and *Gab 3* for 35 cycles. Positive control for PCR amplification was done using hypoxanthine guanine phosphoribosyl transferase (HPRT) primers.

Western blot analysis

IL-2-activated wild-type (WT) or *Gab2*^{-/-} NK cells were used for Western blot analysis. The cells were solubilized in buffer containing 1% Nonidet P-40 (Boehringer Mannheim, Mannheim, Germany), 25 mM Tris HCl, pH 7.5, 150 mM NaCl, 10 mM NaF, 1 mM sodium vanadate, and protease inhibitors from Boehringer Mannheim. After 15 min on ice, the extracts were centrifuged for 30 min at 27,000 g, and supernatants were boiled in Laemmli sample buffer and used for Western blot.

Flow cytometry

Cell suspensions (0.5–1×10⁶ cells/sample) from spleen, peritoneal cavity, liver, and BM were stained with different mAb conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), biotin, or allophycocyanin (APC; from PharMingen, San Diego, CA). Biotin-conjugated mAb were revealed by using streptavidin-peridinin chlorophyll protein (PharMingen). Analysis was performed on a FACSCalibur flow cytometer using Cell Quest software (Becton Dickinson, San Jose, CA).

NK cell cytotoxicity assays

A standard 4-h ⁵¹Cr release assay was used to measure NK activity in vitro. Target cells [yeast artificial chromosome-1 (YAC-1), resistance-modulating agent (RMA), Chinese hamster ovary (CHO)] were labeled with 100 μCi ⁵¹Cr (ICN Pharmaceutical, Costa Mesa, CA). Red cell-depleted splenocytes were used fresh (after passage on nylon wool column to enrich for NK cells) or cultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS), 5 × 10⁻⁵ M β-mercaptoethanol (β-ME), 100 μg/ml streptomycin, 100 U/ml penicillin, and 1000 U/ml human IL-2 (R&D Systems, Minneapolis, MN) for 5–8 days. Before the assay, cells were stained with anti-NK1.1 mAb conjugated to PE or APC and anti-CD3 mAb conjugated to FITC. The number of effector cells was adjusted so as to have equivalent numbers of NK1.1⁺CD3⁻ NK cells in the assay. For ADCC, RMA lymphoma cells were incubated with 20 μg/ml anti-Thy-1 mAb (anti-CD90.2) for 20 min at 4°C prior to the 4-h ⁵¹Cr release assay done using IL-2-activated NK cells as effectors. RMA cells belong to the T cell lineage and originate from C57BL/6 mice and therefore express the CD90.2 Ag. As they express major histocompatibility complex (MHC) class I, they are relatively resistant to NK cell cytotoxicity. Coating RMA cells with anti-CD90.2 makes them susceptible to NK cell-mediated lysis, overcoming the resistance imposed by MHC class I. For PI-3K-inhibition experiments, IL-2-activated NK cells were incubated for 30 min at 37°C with vehicle alone [dimethyl sulfoxide (DMSO)] or with various concentrations of the specific PI-3K inhibitor LY294002 and thereafter, used for the ADCC assay against RMA targets that had been previously coated or not with anti-CD90.2 at 20:1 or for spontaneous cytotoxicity assay against YAC-1 targets at 5:1.

Cytokine production

NK1.1⁺CD3⁻ NK cells were electronically sorted with a FACStar Plus from fresh spleen and were cultured in RPMI 1640 supplemented with 10% FCS and 5×10^{-5} M β -ME in the presence of 1000 U/ml human recombinant IL-2 for 5–6 days. Tissue-culture plates were coated with 20 μ g/ml mAb diluted in phosphate-buffered saline for 16 h at 4°C. NK cells (10^5) were incubated in RPMI 1640 containing 10% FCS and IL-2 (1000–2000 U/ml) and were cultured for 24 or 48 h in plates containing the immobilized antibodies or IL-12 (5 ng/ml). The amount of IFN- γ released in the culture supernatants was determined by using the mouse IFN- γ -specific enzyme-linked immunosorbent assay kit OptEIA (PharMingen) and was used according to the manufacturer's instructions.

Statistical analysis

Student's *t*-test was done using Excel software. $P > 0.05$ was considered statistically nonsignificant.

RESULTS

Gab2 and Gab3 but not Gab1 are expressed in mouse NK cells

The three members of the Gab family are differentially expressed in various tissues and cell types [19]; however, the expression pattern in NK cells is not known. RT-PCR analysis of NK1.1⁺CD3⁻ cells purified from fresh splenocytes revealed that NK cells express Gab2 and Gab3 but not Gab1 mRNA (Fig. 1a). Splenic NK1.1⁺CD3⁻ NK cells activated in vitro for 6–8 days with IL-2 showed a similar pattern of expression (data not shown). Moreover, the absence of Gab2 did not cause qualitative changes in the expression patterns of the various Gab family members. Thus, *Gab2*^{-/-} NK cells, like WT NK cells, expressed Gab3 but not Gab1 transcripts (Fig. 1a).

Gab2 protein was expressed in NK cells as shown by Western blot, although the level of expression was lower than that found in BMM (Fig. 1b) and in mast cells (not shown). Mast cells of *Gab2*^{-/-} mice have been reported to express a truncated version of the Gab2 protein that can, in principle, compensate for at least some functional activities of the full-length Gab2 protein [14]. The sharp band showing in NK cell extract from *Gab2*^{-/-} mice is most probably a cross-reactive one, as no truncated form of Gab2 was detected in NK cells purified

from *Gab2*^{-/-} mice (Fig. 1c). Gab2 is phosphorylated upon engagement of the receptors for IL-2 and IL-15 [22]. IL-2-activated NK cells, unlike resting T cells, presented a migration shift compatible with phosphorylation of Gab2 (data not shown).

NK cell development in *Gab2*^{-/-} mice

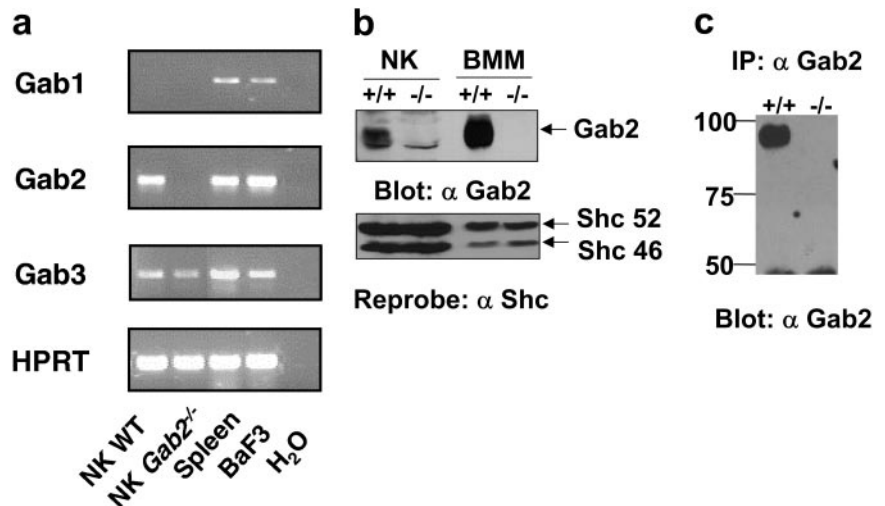
Gab2 participates in the signal-transduction machinery of the receptors for several cytokines including some that are crucial for the biology of NK cells such as stem cell factor, Flt3L, IL-2, and IL-15 (reviewed in refs. [19, 20]). Nevertheless, the percentages and absolute numbers of NK1.1⁺CD3⁻ NK cells in WT and *Gab2*^{-/-} mice were not significantly different in any of the organs analyzed. Thus, NK cell development appears to proceed normally in the absence of Gab2 (Fig. 2a). Moreover, *Gab2*^{-/-} NK cells responded with a vigorous proliferation to IL-2 stimulation in vitro.

NK cell differentiation follows a series of steps that can be identified by studying the combinations of surface markers expressed sequentially on the developing NK cell [20]. WT and *Gab2*^{-/-} NK cells showed comparable expressions of various NK cell-surface markers such as members of the Ly49 and NKG2 families, DX5 (CD49b), Mac-1 (CD11b), 2B4 (CD124), and CD69 (Fig. 2b and data not shown). Thus, NK cells of *Gab2*^{-/-} mice show a mature phenotype. In line with the results obtained with the expression of all the other markers tested, no difference in Fc γ RIII (CD16) expression was found between WT and *Gab2*^{-/-} NK cells (Fig. 2c: MFI=26.3 \pm 3.5 and 23.4 \pm 2.5 in WT and *Gab2*^{-/-}, respectively; $P=0.11$; $n=4$).

PI-3K but not Gab2 is crucial for spontaneous cytotoxicity and ADCC

The signals that activate ADCC are better characterized than those that activate spontaneous cytotoxicity [2, 23]. For example, the PI-3K pathway has been shown to be essential for ADCC in human NK cells [5, 6], but its role in spontaneous cytotoxicity is controversial [2, 5, 9]. We show here that IL-2-activated mouse NK cells depend on functional PI-3K to mediate effective spontaneous cytotoxicity and ADCC (Fig. 3,

Fig. 1. NK cells express Gab2 and Gab3 but not Gab1. (a) Total RNA was extracted from freshly purified or IL-2-activated (6–8 days cultures) WT and *Gab2*^{-/-} NK cells. Total splenocytes and the pre-B cell lymphoma BaF3 cell line, which express all three Gab family members, were used as controls. (b) Whole cell lysates of purified NK cells and BMM from WT (+/+) and *Gab2*^{-/-} (-/-) mice were immunoblotted with anti-Gab2 Ab (upper panel) and anti-Shc antibodies (lower panel) for loading control. (c) Whole cell lysates of 5×10^7 -purified NK cells from WT (+/+) and *Gab2*^{-/-} (-/-) mice were immunoprecipitated (IP) with anti-Gab2 Ab and subsequently immunoblotted with anti-Gab2 Ab.



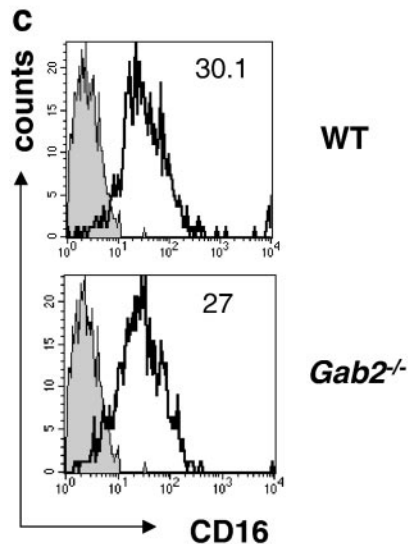
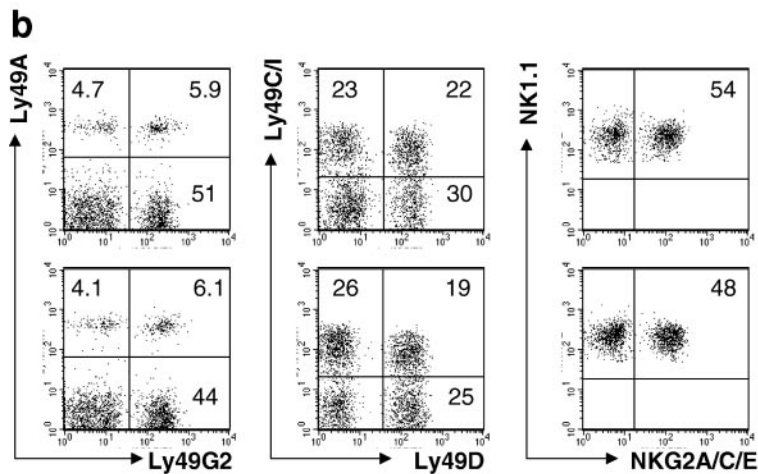
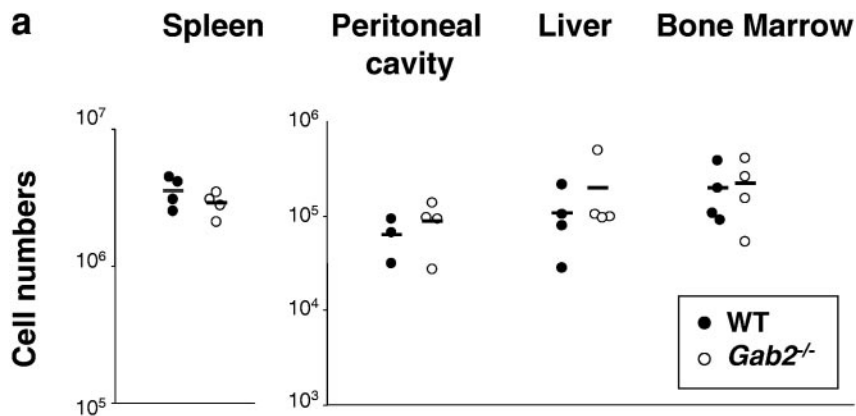


Fig. 2. *Gab2* is dispensable for NK cell development. (a) Cells from spleen, BM, liver, and peritoneal cavity were stained for NK1.1 and CD3. Absolute numbers of NK1.1+CD3⁻ cells were counted. Each data point represents a single mouse. Results are pooled from four independent experiments. (b) Spleen cells were stained with NK1.1-PE and CD3-FITC. Figures show percentages of NK cells (NK1.1+CD3⁻) positive for different NK receptors. No significant differences were detected between WT and *Gab2*^{-/-} NK cells. Results are from one representative of four independent experiments. (c) Spleen cells were stained as described in b. Figure shows mean fluorescence intensity (MFI) of CD16 expression in NK1.1+CD3⁻ NK cells. No significant difference was detected between WT and *Gab2*^{-/-} NK cells. Results are from one representative of four independent experiments.

a and b). In addition, NK cells of mice lacking an isoform of the catalytic subunit of PI-3K had defective FcR responses upon Ab cross-linking, directly implicating PI-3K downstream of FcR in mouse NK cells (S. Zompi and F. Colucci, unpublished observation), recapitulating findings in human NK cells [6]. As *Gab2* plays crucial roles in PI-3K activation downstream of FcεR in mast cells and of FcγR in macrophages, it could play similar roles downstream of NK cell-activating receptors. However, *Gab2*^{-/-} NK cells could spontaneously kill YAC-1 lymphoma cells as efficiently as WT NK cells (Fig.

4a); thus, *Gab2* is not essential for this PI-3K-dependent NK cell function. Moreover, ADCC activity of *Gab2*^{-/-} NK cells was comparable with that of WT NK cells (Fig. 4b). Thus, in contrast to mast cells [14] and macrophages [15], the multichain immune-recognition FcR expressed by NK cells is functional in the absence of *Gab2*.

Finally, we tested and excluded the possibility that the Ly49D-activating receptor could require *Gab2*, as cytotoxicity against CHO target cells was similar in WT and *Gab2*^{-/-} NK cells (data not shown).

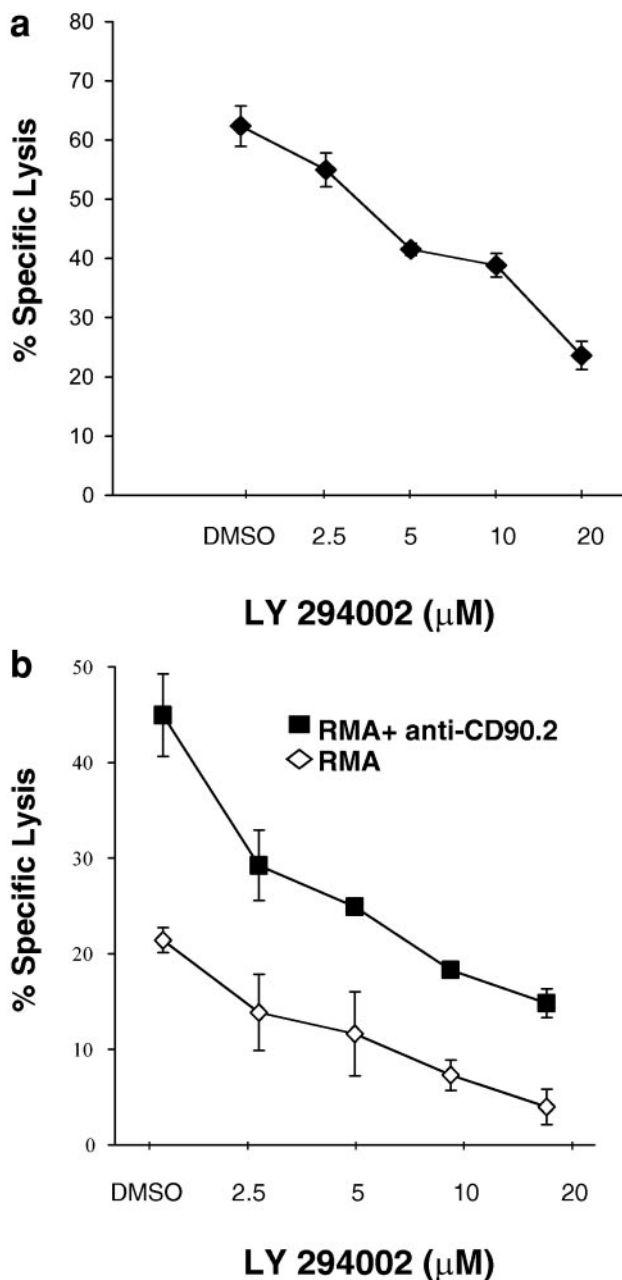


Fig. 3. Spontaneous cytotoxicity and ADCC depend on PI-3K. (a) Spontaneous cytotoxicity: IL-2-activated NK cells from *Rag2*^{-/-} mice were first incubated with the indicated doses of the PI-3K inhibitor LY294002 and thereafter, coincubated with YAC-1 tumor cells for a 4-h chromium release assay at an effector-to-target ratio of 5:1. Data are means \pm SD from a representative of three independent experiments that gave similar results. (b) ADCC: IL-2-activated NK cells from *Rag2*^{-/-} mice were first incubated with the indicated doses of the PI-3K inhibitor LY294002 and thereafter, coincubated with RMA tumor cells that had been previously coated (■) or not (◇) with anti-CD90.2, for a 4-h chromium release ADCC assay at an effector-to-target ratio of 20:1. Data are means \pm SD from a representative of three independent experiments that gave similar results.

IFN- γ production in the absence of Gab2

NK cells produce IFN- γ upon stimulation by proinflammatory cytokines IL-12 and IL-18 or upon stimulation of different receptors, including CD16. These physiological situations can be reproduced in vitro by stimulating NK cells with natural

ligands such as IL-12 or plate-bound antibodies. *Gab2*^{-/-} NK cells could produce as much IFN- γ as WT NK cells in response to all the different stimuli tested, including IL-12, anti-CD16, anti-2B4, and anti-NK1.1 Ab (**Fig. 5**). Similar results were obtained by stimulating NK cells with nonsaturating doses of anti-CD16, (data not shown). Therefore, cytokine production by NK cells is not perturbed in the absence of Gab2.

DISCUSSION

We found that NK cells express the scaffolding proteins Gab2 and Gab3, albeit Gab2 was expressed at lower levels compared with macrophages and mast cells. NK cells did not express Gab1 transcripts. Gab2 plays a pivotal role in Fc ϵ R-initiated, allergic responses in mast cells [14] and Fc γ R-mediated phagocytosis in macrophages [15]. Moreover, Gab2 has been implicated in lymphocyte signal transduction, where it mediates a negative control over T cell receptor signaling [24] and transduces signals downstream of the receptors for IL-2 and IL-15 (IL-2R and IL-15R, respectively) in T cells [22]. In striking contrast, our results show that Gab2 is not an essential player in the transduction of crucial signals that regulate NK cell development and differentiation, nor NK cell functions, including expansion and activation in IL-2, spontaneous cytotoxicity, Fc γ R-mediated ADCC, and cytokine production. *Gab2*^{-/-} NK cells do not express the truncated form of Gab2 found in *Gab2*^{-/-} mast cells, ruling out any possible contribution to functional activity.

The lack of Gab1 transcripts in NK cells and the absence of an obvious phenotype in *Gab2*^{-/-} NK cells make Gab3 the most likely family member involved in the biology of NK cells. Normal numbers of DX5⁺Mac-1⁺ NK cells were found in spleen and BM of *Gab3*^{-/-} mice [25], indicating that Gab3 may also be dispensable for NK cell development. However, NK cell functions were not assessed in *Gab3*^{-/-} mice. The analysis of NK cell functions in *Gab3*^{-/-} mice combined with the analysis of NK cell development and functions in compound *Gab2*^{-/-}*3*^{-/-} mice will address the questions of whether Gab scaffolding proteins are required for NK cell biology; if so, which aspects of NK cell development and functions they regulate; and whether Gab2 and Gab3 are functionally redundant.

From our results stem a number of considerations about the differential requirements for Gab2 in NK cells as compared with other leukocytes, including T lymphocytes, macrophages, and mast cells. First, stimulation of IL-2R and IL-15R in human T and NK cells induces Gab2 phosphorylation, which is Src-independent and Janus tyrosine kinase-3-dependent [22]. IL-2R and IL-15R, in human and mouse, share the $\beta\gamma$ signaling complex but express unique high-affinity α -chains. Mouse NK cells, unlike human NK cells, do not express the high-affinity α -chain of the IL-2R; therefore, NK cell responses to IL-2 are actually mediated by the IL-15 $\beta\gamma$ receptor complex. As *Gab2*^{-/-} NK cells expanded normally and became fully activated following IL-2 stimulation in vitro, our results suggest that Gab2 is not essential for transducing signals downstream of IL-15R, which is a crucial cytokine for NK cell development

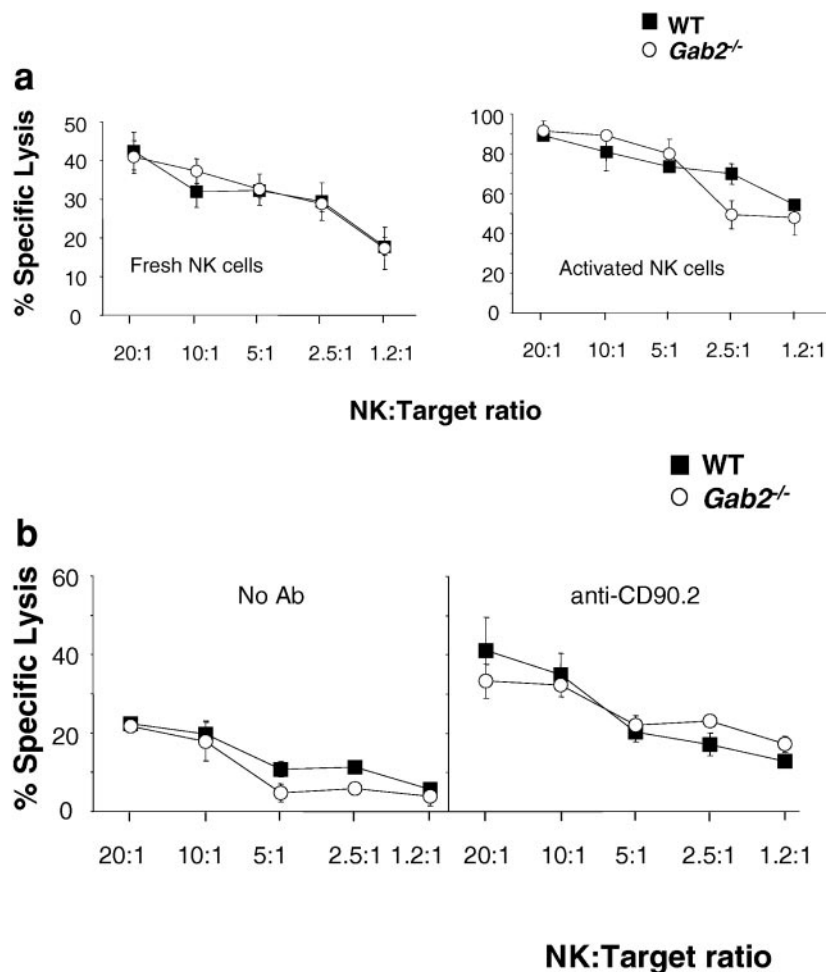


Fig. 4. Spontaneous cytotoxicity and ADCC do not depend on Gab2. (a) Spontaneous cytotoxicity: NK cell-enriched, fresh splenocytes (left panel) or IL-2-activated NK cells (right panel) from WT (control, ■) and *Gab2*^{-/-} mice (○) were used in a 4-h chromium release assay at the indicated NK:target cell ratios against YAC-1 targets. Data are means ± SD from a representative of three independent experiments that gave similar results. (b) ADCC: IL-2-activated WT (control, ■) and *Gab2*^{-/-} (○) NK cells (day 8 cultures) were coincubated with RMA tumor cells that had been previously coated or not with anti-CD90.2 for a 4-h chromium release ADCC assay at the indicated NK:target cell ratios. Data are means ± SD from a representative of three independent experiments that gave similar results.

[20]. The result showing that *Gab2*^{-/-} NK cells develop to normal numbers and display normal expression of differentiation markers is in line with the notion that Gab2 is not essential for IL-15R signaling in NK cells. In this context, it is worth noting that our results suggest that Gab2 is not required to transduce signals downstream of other cytokine receptors involved in NK cell development such as c-kit [26] and FLT3

[27], in spite of the association of Gab2 with these receptors (ref. [19] and references therein). In fact, Gab2 is required for c-kit signaling during mast cell development [28] but not NK cell development (this report).

Second, we found that signal transduction downstream of the NK cell FcγR was apparently intact in *Gab2*^{-/-} NK cells, whereas macrophages FcγR and mast cells FcεR require Gab2 to function properly. However surprising this finding was, other instances of such cell-specific requirement downstream of FcγR have been described previously. For example, although Src, Syk, and PI-3K kinases participate in the activation cascades that trigger FcγR-mediated phagocytosis in macrophages and FcγRIII-mediated ADCC in NK cells (refs. [5, 17, 18] and this report), different members of these kinase families may be involved in these signaling pathways. Thus, FcγR preferentially associates with Lyn and Syk in macrophages, whereas it couples with Lck and ZAP-70 in NK cells (reviewed in ref. [16]). One possibility is that NK cell FcγR preferentially uses Gab3, whereas macrophages use Gab2. Future experiments with *Gab3*^{-/-} NK cells will clarify this issue.

Phagocytosis and ADCC engage different cellular events, i.e., formation of phagocytic cup versus degranulation of cytotoxic granules. Although the same FcγR triggers these two effector functions, separate pathways and distinct signaling molecules may well regulate different downstream events. Conversely, FcεR and FcγR induce similar downstream events in

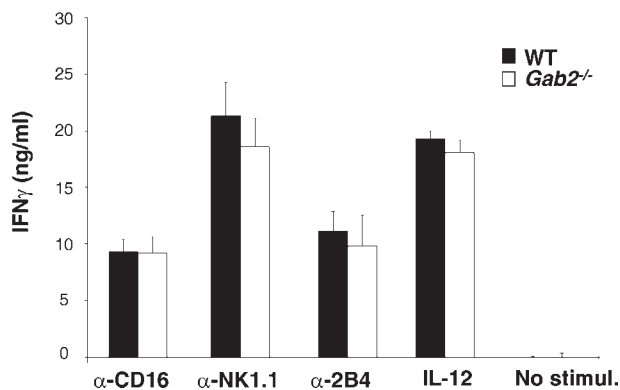


Fig. 5. Gab2 is dispensable for IFN-γ production. Sorted IL-2-activated NK cells of *Gab2*^{-/-} and control WT mice were stimulated with 5 ng/ml IL-12, 20 μg/ml anti-CD16, anti-NK1.1, and anti-2B4 or were left with no stimulus. Data are means ± SD of triplicates from one representative of two independent experiments giving similar results.

mast cells and NK cells, respectively, i.e., degranulation and cytokine gene transcription. Given that, it was striking to find intact ADCC and cytokine release in *Gab2*^{-/-} NK cells, as opposed to defective degranulation and cytokine release in *Gab2*^{-/-} mast cells [14]. *Gab2* and *Gab3* may be functionally redundant in NK cells, or NK cells preferentially use *Gab3*. Alternatively, the pathways that regulate cytokine induction downstream of FcRs are distinct in mast cells and NK cells.

The activating receptor NKG2D has been shown to be a major contributor to trigger NK cell-spontaneous cytotoxicity against YAC-1 targets. In fresh, nonstimulated NK cells, this receptor is constitutively associated with the adaptor DAP10, which lacks ITAM but possess a consensus motif for recruitment and activation of PI-3K. Our results confirm that PI-3K is a crucial component of spontaneous cytotoxicity against YAC-1 tumor cells and suggest that *Gab2* is not essential for the NKG2D–DAP10–PI-3K pathway of spontaneous cytotoxicity.

Finally, our results may have some clinical implications. Mast cell-deficient mice display FcεR-independent allergic responses [29], and *FcγRIII*^{-/-} mice display reduced IgE-mediated anaphylaxis [30]. Therefore, cells other than mast cells and receptors other than FcεR can participate in IgE-mediated anaphylaxis. IgE can activate FcγRIII in NK cells and initiate production of cytokines and chemokines [31]. This suggests a possible involvement of NK cells in type I hypersensitivity. In line with this, NK cells regulate allergen-induced eosinophilic airways inflammation [32]. In addition, IgE-mediated ADCC could also play a role in type II hypersensitivity. Pharmacological targeting of *Gab2* in mast cells is a potential strategy to treat allergy; however, our results suggest prudence, as NK cells may participate in IgE-mediated anaphylaxis in a *Gab2*-independent manner.

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