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Dissecting NK Cell Development Using a Novel Alymphoid Mouse Model: Investigating the Role of the c-*abl* Proto-Oncogene in Murine NK Cell Differentiation[1]

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NK lymphocytes participate in both innate and adaptive immunity by their prompt secretion of cytokines including IFN-γ, which activates macrophages, and by their ability to lyse virally infected cells and tumor cells without prior sensitization. Although these characteristics of NK cells are well documented, little is known about the genetic program that orchestrates NK development or about the signaling pathways that trigger NK effector functions. By crossing NK-deficient common γ-chain (γ_c) and recombinase activating gene (RAG)-2 mutant mice, we have generated a novel alymphoid (B^-, T^-, and NK^-) mouse strain (RAG2/γ_c) suitable for NK complementation in vivo. The role of the c-*abl* proto-oncogene in murine NK cell differentiation has been addressed in hemopoietic chimeras generated using RAG2/γ_c mice reconstituted with c-$abl^{-/-}$ fetal liver cells. The phenotypically mature NK cells that developed in the absence of c-*abl* were capable of lysing tumor targets, recognizing "missing self," and performing Ab-dependent cellular cytotoxicity. Taken together, these results exclude any essential role for c-*abl* in murine NK cell differentiation in vivo. The RAG2/γ_c model thereby provides a novel approach to establish a genetic map of NK cell development. *The Journal of Immunology,* **1999, 162: 2761–2765.**

The c-*abl* proto-oncogene is the cellular homologue of the Abelson leukemia virus. c-*abl* encodes a highly conserved (1) intracellular protein tyrosine kinase having both DNA- (2) and actin-binding domains (3). Targets of the c-Abl kinase include the Rb gene product, RNA polymerase II, Crk, and the JNK pathway (4), and, as such, c-Abl has been proposed to control cell growth (5, 6), although it is not clear whether it acts in a positive or negative fashion (7). c-*abl* is expressed ubiquitously and throughout development, but is more abundantly expressed in the spleen, thymus, and testes (8). Mice deficient in c-*abl* develop to the perinatal period but generally fail to thrive, runt, and die around 3 wk of age (9, 10). Although the mechanism underlying the severe phenotype of c-*abl* mutant mice has not been elucidated, an effect of c-*abl* deficiency on lymphoid development has been suspected, because c-$abl^{-/-}$ mice develop a generalized lymphopenia and appear highly susceptible to infections (9–12). While mature T and B cells in c-$abl^{-/-}$ mice appear functional (9–12), a defect in NK cell differentiation could predispose c-$abl^{-/-}$ mice to infection. Mature, functional NK cells first appear in the mouse at 3 wk of age (13, 14), coincident with the demise of most c-*abl*−/− mice. As such, any potential role of c-*abl* in NK cell physiology and in the increased susceptibility of c-$abl^{-/-}$ mice to pathogens could not be addressed.

The recombinase activating gene (RAG)[3]-2 blastocyst complementation system introduced by Alt and colleagues has proved instrumental in defining the potential role in T and B cell development of genes that give rise to embryonic lethality (15, 16). Therefore, this approach can discriminate cell-intrinsic defects from in *trans* effects for any given mutation. Nevertheless, this system cannot be used to study gene effects on NK development, because RAG2-deficient mice develop NK cells (17), thereby precluding the possibility of studying the donor-derived NK cells in the absence of competing mature host-derived cells. While other mouse models of NK cell deficiency are available, they are not satisfactory because they either rely on temporary Ab depletion of pre-existing NK cells (18) or they are associated with major defects in lymphopoiesis, lymphoid homeostasis, or autoimmunity (19–21).

We have developed a novel alymphoid mouse strain that combines the NK-deficiency found in common γ-chain (γ_c; a shared component of the receptors for IL-2, -4, -7, -9, and -15) mutant mice with the T and B cell block due to mutation in the RAG2 gene (RAG2/γ_c double mutant mice). RAG2/γ_c mice offer certain advantages over RAG2 mice for studies involving lymphoid reconstitution and are well suited for the in vivo study of NK cell differentiation. In this report, we demonstrate the feasibility and utility of the RAG2/γ_c mouse model by examining the role of the c-*abl* proto-oncogene in NK cell development.

Materials and Methods

Mice

Mice with a null mutation in the γ_c (20) were from the fourth generation backcross to the C57BL/6 background. RAG2 mice (17) from the tenth

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[3] Abbreviations used in this paper: RAG, recombinase activating gene; ADCC, Ab-dependent cellular cytotoxicity; A-LAK, adherent lymphokine-activated killer; BM, bone marrow; γ_c, common cytokine receptor γ-chain; FL, fetal liver; HSC, hemopoietic stem cell; NWNA, nylon wool nonadherent.

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Table I. *Lymphoid cellularity in reconstituted RAG2/γ_c mice*[a]

Mouse	BM Cell No. ($\times 10^6$)	Lymphoid Cells (range in %)	Thymus Cell No. ($\times 10^6$)	Lymphoid Cells (range in %)	Spleen Cell No. ($\times 10^6$)	Lymphoid Cells (range in %)
C57BL/6	12.5 ± 1.6 ($n = 4$)	25–30	144 ± 22 ($n = 5$)	81–86	89.2 ± 11 ($n = 6$)	67–74
RAG2/γ_c, c-*abl*$^+$	11.9 ± 1.4 ($n = 4$)	13–27	46.3 ± 28 ($n = 6$)	81–84	91.0 ± 23 ($n = 6$)	64–70
RAG2/γ_c, c-*abl*$^{-/-}$	10.4 ± 2.4 ($n = 4$)	14–30	58.1 ± 40 ($n = 6$)	79–85	84.2 ± 15 ($n = 6$)	62–71
RAG2$^-$	7.7 ± 0.5 ($n = 4$)	10–15	2.3 ± 0.4 ($n = 5$)	30–50	4.4 ± 1.2 ($n = 5$)	25–40
RAG2/γ_c	7.2 ± 0.3 ($n = 4$)	2–10	0.04 ± 0.01 ($n = 5$)	5–20	1.2 ± 0.1 ($n = 4$)	10–20

[a] Red cell-depleted cell suspensions were enumerated from each organ of the indicated mice, and the percentages of lymphoid cells were measured by flow cytometry on the basis of forward and side scatter parameters.

generation backcross to C57BL/6 were kindly provided by B. Rocha (Institut National de la Santé et de la Recherche Médicale, Unite 345, Paris, France). C57BL/6 and β_2-microglobulin-deficient C57BL/6 mice were obtained from Centre de Développement des Techniques Avancées/Centre National de la Recherche Scientifique (Orleans, France). Mice doubly deficient in RAG2 and γ_c (RAG2/γ_c) were obtained by intercrossing, and genotypes were determined by PCR on DNA derived from tail snips (primer sequences available from the authors). RAG2/γ_c mice older than 6 wk of age were used as recipients for lymphoid reconstitution. Mice heterozygous for the c-*abl* mutation (c-*abl*$^{+/-}$; Ref. 9), which had been backcrossed >10 generations onto C57BL/6, were crossed with c-*abl*$^{+/-}$ mice backcrossed for 5 generations onto 129/Sv to generate day 18 c-*abl*$^{-/-}$ and control (c-*abl*$^{+/+}$ or c-*abl*$^{+/-}$) embryos. The morning of the vaginal plug discovery was designated as day 0. The c-*abl* genotypes of the embryos were determined by Southern blotting as described (9).

Generation of hemopoietic chimeras

Pregnant female mice were sacrificed and the embryos were explanted under sterile conditions. Fetal liver (FL) cell suspensions were obtained by passage of the tissue through a 23-gauge needle. RAG2/γ_c mice were irradiated with 0.3 Gy from a cobalt source and 4 h later were injected i.v. with 5×10^6 FL cells as a source of hemopoietic stem cells (HSC). No differences were noted between reconstitutions made with c-*abl*$^{+/+}$ and c-*abl*$^{+/-}$ FL-HSCs, which will be referred to as c-*abl*$^+$. Secondary transfers using bone marrow (BM) cells of FL-HSC-reconstituted RAG2/γ_c mice were performed by i.v. injection of 10^7 total BM cells into irradiated (0.3 Gy) RAG2/γ_c recipients. All mice received tetracycline and bactrim in the drinking water for the period following the transfer.

Flow cytometry

Single-cell suspensions were prepared from spleen, BM, thymus, and liver. Erythrocytes were lysed in ammonium chloride, and cells were resuspended in PBS with 3% FCS and 0.01% sodium azide. mAbs directly conjugated to FITC, phycoerythrin, Tricolor (TRI), or biotin were used for immunofluorescence analysis, including CD2, CD3, CD4, CD8, TCR$\alpha\beta$, TCR$\gamma\delta$, CD11b, CD16 (FcγRII/III), CD19, CD24, CD45R (B220), IgM, CD90 (Thy-1), CD117 (c-*kit*), CD122 (IL2Rβ), CD132 (γ_c), and the NK markers CD161 (NK1.1), DX5, Ly49A, Ly49C/I, and Ly49G2 (PharMingen, San Diego, CA). Biotin conjugates were revealed by streptavidin-TRI (Caltag, South San Francisco, CA). Cells (10^5–10^6) were first incubated with anti-CD16 and then stained with a mixture of biotinylated and fluorochrome-labeled mAbs at saturating concentrations, washed twice, and finally incubated with streptavidin-TRI. Analysis was performed on a FACScan flow cytometer using Lysis II software (Becton Dickinson, San Jose, CA). Dead cells were excluded by their forward and side scatters parameters, and an electronic gate was set to acquire (5–10 $\times$ 10^3) lymphoid cells.

Generation of IL-15-activated NK cell cultures

Splenocytes were passed through nylon wool columns to remove B cells and macrophages. Nylon wool nonadherent (NWNA) cells were cultured in flat-bottom 24-well plates at 5×10^6 cells/ml in complete medium (RPMI 1640 with 10% FCS, 10^{-5} M β-ME, 100 μg/ml streptamycin, and 100 U/ml penicillin), supplemented with 0.5 μg/ml of human IL-15 (R & D Systems, Minneapolis, MN). After 3–4 days, the nonadherent cells were removed, and the adherent lymphokine-activated killer (A-LAK) cells were refed and cultured until day 8–10. A-LAK cultures produced in this manner routinely contained >95% NK1.1$^+$/CD3$^-$ cells.

In vitro cytotoxicity

A ^{51}Cr release assay was used to measure NK activity in vitro as described (22). Target cells (YAC-1, EL-4, or ConA-activated blasts) were labeled with 100 μCi ^{51}Cr (ICN Pharmaceutical, Costa Mesa, CA), and 5×10^3 targets were incubated with graded numbers of effector cells in 200 μl of medium for 4 h. For Ab-dependent cellular cytotoxicity (ADCC), day 8–10 A-LAK cells were used as effectors, and targets were EL-4 cells coated with anti-CD90 (Thy-1.1) mAb. Radioactivity released into the cell-free supernatant was measured, and the percentage of specific lysis was calculated as following: 100 $\times$ (experimental release $-$ spontaneous release/maximum release $-$ spontaneous release). The spontaneous release never exceeded 15%.

In vivo NK cell-mediated tumor rejection

Recipient mice (H-2^b) were injected s.c. (10^5 cells/0.1 ml) with class I-deficient RMA-S cells (23). Mice were monitored for palpable tumors and were sacrificed when the tumor mass reached 15 mm in diameter. In this assay, NK-deficient mice succumb to tumors within 15 days, while C57BL/6 control mice as well as RAG2$^{-/-}$ mice do not develop tumors over the period of observation (>60 days).

Results

Generation and characterization of RAG2/γ_c mutant mice

A novel alymphoid mouse strain was generated by intercrossing RAG2-deficient (17) and γ_c-deficient mice (20). RAG2/γ_c mutant mice were viable and bred normally under pathogen-free conditions. We characterized lymphoid development in RAG2/γ_c vs RAG2 or control C57BL/6 mice. In contrast to RAG2 mice, RAG2/γ_c mutants were almost completely depleted in early lymphoid precursors (Table I and Fig. 1*A*). For example, CD19$^+$ BM cells were almost completely absent in RAG2/γ_c mice, and thymic cellularity was further reduced 100-fold compared with RAG2

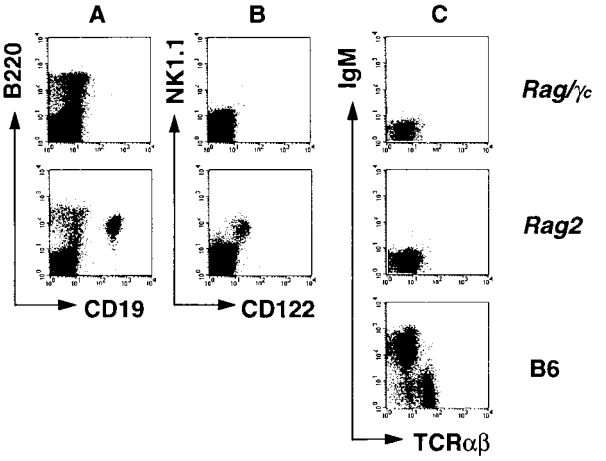

FIGURE 1. RAG2/γ_c mice have fewer lymphoid precursors than RAG2 mice and lack mature NK, B, and T lymphocytes. *A*, Compared with RAG2 mutant mice, BM cells from RAG2/γ_c mice have only background levels of CD19$^+$B220$^+$ B lineage committed precursors, while the CD19$^-$B220$^+$ subset can be detected. *B*, NK cells (CD122$^+$NK1.1$^+$) are undetectable in RAG2/γ_c BM cells, while they make up a significant fraction in RAG2 mice. *C*, RAG2/γ_c and RAG2 spleen cells are completely devoid of mature B (IgM$^+$) and $\alpha\beta^+$ T cells.

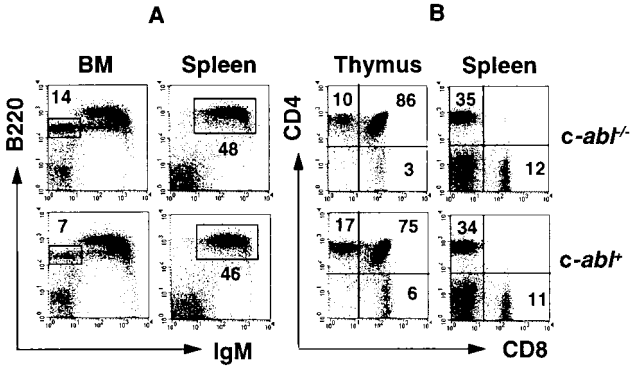

FIGURE 2. Normal development of B and T lymphocytes in the absence of c-*abl*. *A*, B cell development. c-*abl*$^{-/-}$ chimeras display normal frequencies of IgM$^-$B220$^+$ BM cells (boxed) and IgM$^+$B220$^+$ mature B cells (boxed) in the spleen. *B*, T cell development. The four major thymocyte subsets (CD4$^-$CD8$^-$, CD4$^+$CD8$^-$, CD4$^+$CD8$^+$, and CD4$^-$CD8$^+$) and percentages of splenic CD4$^+$ and CD8$^+$ T cells were comparable in c-*abl*$^{-/-}$ and c-*abl*$^+$ chimeras. Staining is representative of four independent experiments.

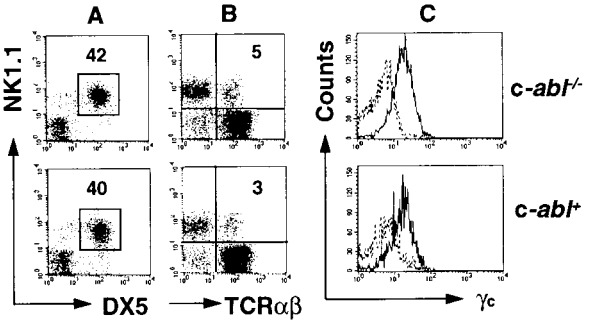

FIGURE 3. NK development in c-*abl* hemopoietic chimeras. Splenic and hepatic lymphoid cells from RAG2/γ_c chimeras were isolated and stained with the indicated Abs. *A*, An electronic gate was set to exclude CD19$^+$ B cells and $\alpha\beta^+$ T cells. A population of DX5$^+$/NK1.1$^+$ splenic NK cells (boxed areas) can be observed in both c-*abl*$^{-/-}$ and c-*abl*$^+$ chimeras. *B*, IgM$^-$ liver lymphocytes of both c-*abl*$^{-/-}$ and c-*abl*$^+$ chimeras contain NK1.1$^+$/TCR$\alpha\beta^+$ NK-T cells and NK1.1$^+$/TCR$\alpha\beta^-$ NK cells. *C*, Liver NK1.1$^+$ cells were stained with anti-γ_c mAb (unbroken line) or isotype-matched control mAb (dotted line). All NK cells were found to be donor derived. Staining is representative of four independent experiments.

mice (10,000-fold fewer thymocytes than controls). The effect of the γ_c mutation on the development of these early lymphoid precursors likely reflects their requirement for IL-7/IL-7Rα/γ_c signaling for survival (reviewed in Ref. 24). As expected, no mature IgM$^+$ B cells or $\alpha\beta^+$ T cells were found in mice harboring the RAG2 mutation (Fig. 1*C*). In addition, RAG2/γ_c mice completely lacked NK1.1$^+$ cells in the BM (Fig. 1*B*) and spleen (data not shown). The presence of functional NK cells lacking the NK1.1 marker in RAG2/γ_c mice appeared unlikely based on the following criteria: 1) splenocytes from RAG2/γ_c mice show no natural cytotoxicity against YAC-1 targets in vitro at E:T ratios of 300:1, 2) RAG2/γ_c mice fail to augment IFN-γ blood levels following administration of murine IL-12 in vivo, and 3) RAG2/γ_c mice fail to reject allogeneic BM grafts or MHC class I-deficient tumors in vivo (data not shown). In contrast, RAG2 mice retain these three NK cell effector functions (Ref. 17 and data not shown). Taken together, these results demonstrate that RAG2/γ_c mice have no mature B, T, or NK cells and are therefore a suitable host for in vivo NK complementation.

*Lymphoid reconstitution of RAG/γ_c mice using c-*abl$^{-/-}$ *HSC*

We hypothesized that a defect in NK cell function could contribute to the increased susceptibility of c-*abl*$^{-/-}$ mice to infections (9, 10). Most c-*abl*$^{-/-}$ mice succumb at 3 wk of age, which is coincident with the first appearance of functional NK cells in the mouse (13, 14). To investigate the role of c-*abl* in NK development, we generated hemopoietic chimeras in irradiated (0.3 Gy) RAG2/γ_c mice using FL cells from c-*abl*$^+$ and c-*abl*$^{-/-}$ embryos. Serial blood sampling demonstrated lymphoid reconstitution beginning at 4 wk posttransfer, which normalized by 8 wk posttransfer (data not shown). At this point, chimeras were sacrificed, and lymphoid development was assessed in the BM, thymus, spleen, and liver. In chimeras injected with c-*abl*$^+$ FL cells, full lymphoid reconstitution was observed. Normal absolute numbers of thymic and splenic lymphocytes were found, representing increases of 100- to 10,000-fold compared with nonmanipulated RAG2/γ_c mice (Table I). The appearance of mature T and B cells in the chimeras (Fig. 2) demonstrated that donor-derived precursors could fully differentiate in this setting, and the donor origin of the resultant NK cells was confirmed by staining with anti-γ_c mAb (Fig. 3*C*). Thus, all mature lymphoid subsets (B, T, and NK) could be generated from wild-type FL cells following injection into irradiated RAG2/γ_c mice. These results demonstrate that the lymphoid defects in RAG2/γ_c mice are cell-intrinsic and that expression of the γ_c in BM stromal or gut epithelial cells is not required for normal lymphoid development.

The effect of c-*abl*-deficiency on B, T, and NK cell development in RAG2/γ_c chimeras was then assessed. The absolute numbers of lymphoid cells in the BM, thymus, and spleens of c-*abl*$^{-/-}$ chimeras were similar to that of control chimeras (Table I). Moreover, no obvious differences in the development of phenotypically defined lymphoid subsets could be discerned between the two groups of chimeras. Similar percentages and absolute numbers of NK1.1$^+$CD3$^-$ NK cells, CD4$^+$ or CD8$^+$ $\alpha\beta$ T cells, $\gamma\delta$ T cells, NK1.1$^+$ $\alpha\beta$ T cells, B220$^+$IgM$^+$ B cells, and B220$^+$IgM$^-$ pre-B cells were found in the lymphoid organs of c-*abl*$^{-/-}$ and control RAG2/γ_c chimeras (Fig. 2, *A* and *B*, Fig. 3, *A* and *B*, and data not shown). Moreover, the absolute numbers of most lymphocytes subsets were also comparable in chimeric animals to normal C57BL/6 mice, although the development of thymic and peripheral NK1.1$^+$ $\alpha\beta$ T cells were slightly reduced in both c-*abl*$^{-/-}$ and control chimeras (Fig. 3*B*).

*Phenotype and function of c-*abl$^{-/-}$ *NK cells*

A more detailed analysis of the NK cells generated in c-*abl*$^{-/-}$ RAG2/γ_c chimeras was performed. Using a panel of Abs detecting Ags expressed by NK cells, a normal percentage and expression level of CD2, CD11b, CD16, CD45R, DX5, CD122, CD90, and CD117 was found on c-*abl*$^{-/-}$ NK1.1$^+$CD19$^-$TCR$\alpha\beta^-$ spleen cells compared with control NK cells (data not shown). In particular, the expression levels of the inhibitory receptors of the Ly49 family (Ly49A, Ly49C/I, and Ly49G2) and the frequencies of these different Ly49$^+$ NK "subsets" in c-*abl*$^{-/-}$ RAG2/γ_c chimeras was normal (data not shown).

The lytic capacity of freshly isolated splenic c-*abl*$^{-/-}$ NK cells was tested in vitro. c-*abl*-deficient NWNA spleen cells demonstrated normal levels of natural cytotoxicity against YAC-1 thymoma targets (Fig. 4*A*). Day 8–10 A-LAK cells also mediated efficient lysis of YAC-1 targets, as well as Ab-mediated cell cytotoxicity against Ab-coated EL-4 cells (Fig. 4, *B* and *C*). Moreover, both c-*abl*$^+$ and c-*abl*$^{-/-}$ A-LAK cells could discriminate between class I-negative and class I-positive ConA-activated

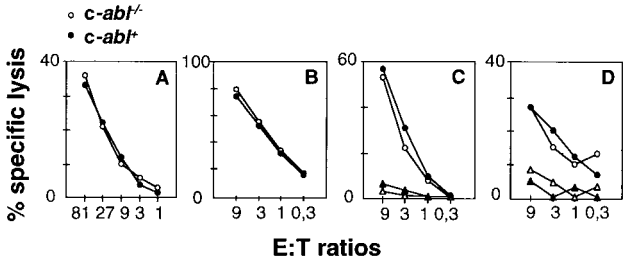

FIGURE 4. Cytotoxic activity of c-*abl*-deficient NK cells. *A*, Freshly isolated splenocytes from poly (I:C)-injected mice were enriched for NK cells by passing over nylon wool, and nonadherent cells (containing about 15% NK1.1$^+$TCR$\alpha\beta^-$ cells) were used as effectors at the indicated ratios. *B–D*, 8–10 day A-LAK cells were generated in vitro using IL-15 and were tested for their ability to lyse YAC-1 targets (*B*), Ab-coated EL-4 targets (*C*, circles), or ConA-activated β_2-microglobulin blasts (*D*, circles) at the indicated E:T ratios. Background levels of lysis are shown for EL-4 cells (*C*, triangles) and for ConA-activated blasts (*D*, triangles, C57BL/6 spleen cells). Each killing assay shown is representative of three independent experiments.

blasts, lysing the former but not the latter (Fig. 4*D*). Taken together, these results suggest that c-*abl* function is not required 1) for NK cell development and maturation, 2) for NK cell recognition and inhibition by self-MHC, and 3) for NK cell receptor calibration in vivo (reviewed in Ref. 25).

Previous studies have demonstrated that c-*abl*-deficient T and B cells have abnormal responses to mitogenic stimulation in vitro (12). In contrast, NK cells from both c-*abl*$^{-/-}$ and c-*abl*$^+$ RAG2/γ_c chimeras were capable of in vitro expansion in response to IL-15. In addition, the A-LAK cultures generated in this fashion were IL-12 responsive (data not shown), ruling out any essential role of c-*abl* in the mitogenic responses of NK cells to IL-12 or IL-15.

To investigate in vivo NK cell function, we examined whether c-*abl* RAG2/γ_c chimeric animals could eliminate MHC class I-negative tumor cells. Mice were injected s.c. with Tap1-deficient (RMA-S) cells (26), and tumor formation was monitored. Both c-*abl*$^+$ and c-*abl*$^{-/-}$ RAG2/γ_c chimeras were able to control growth of MHC class I$^-$ tumor cells (data not shown), demonstrating normal NK lytic activity in vivo.

Discussion

In this report, we describe a novel alymphoid mouse strain harboring the RAG2 and γ_c mutations (RAG2/γ_c). Although a number of immunodeficient mouse models exist (such as *beige*, *nude*, *scid*, RAG, *xid*, and combinations thereof), RAG2/γ_c mice offer considerable advantages over these strains including 1) a complete absence of mature T, B, and NK cells, 2) a stable immunophenotype, 3) no increased propensity to spontaneous tumor formation, and 4) no autoimmune phenomena due to defective lymphoid homeostasis. The alymphoid nature of RAG2/γ_c mice will permit the construction of mice with defined immune systems, which should prove useful in further defining the role of different lymphoid subsets (NK, NK-T, $\gamma\delta$ T, and $\alpha\beta$ T cells) during immune responses to infectious pathogens. The immunodeficiency in RAG2/γ_c mice permits stable hemopoietic engraftment (using either FL or adult BM HSCs) across classical histocompatibility barriers and without irradiation (our unpublished observations). We have recently found that RAG2/γ_c mice accept human PBL xenografts to a similar extent as nonobese diabetic/SCID mice (27). Thus, RAG2/γ_c mice should be useful for a number of applications in lymphoid development, immune responses, tumor immunology, and xenotransplantation.

Alt and colleagues revolutionized the analysis of genes involved in T and B cell development by introducing the RAG2 blastocyst complementation system (15). This powerful technique has been used extensively to study the function of "embryonic lethal" genes in the immune system (reviewed in Ref. 16). Despite this major advance, RAG2 complementation cannot be used to study NK differentiation (because RAG-deficient mice have NK cells; Ref. 17) or early lymphoid precursors (as these develop normally in the absence of RAG genes). The early precursors in RAG2 mice in principle could compete with the mutant donor cells, thereby blocking their development and giving the impression that a given gene is essential for T and/or B cell development. Because RAG2/γ_c mice are also severely depleted in T and B lymphocyte precursors compared with their RAG2 counterparts (Fig. 1), they should offer less competition at these early stages of lymphoid development. Consistent with this hypothesis, we have recently found that c-*kit* deficient (*W/W*) FL cells can give rise to normal numbers of T lymphocytes when grafted in RAG2/γ_c mutant mice (our unpublished observations), whereas the same experiment performed in RAG2-deficient mice failed to generate T lineage cells (28).

In this report, we have used RAG2/γ_c mice to assess the role of the c-*abl* protein tyrosine kinase in NK development. Our results rule out an essential role for c-*abl* in NK differentiation in vivo. In the absence of c-*abl*, normal numbers of NK cells can develop in a phenotypically normal fashion and acquire a lytic capacity for a variety of targets in vitro and in vivo. The proper expression of inhibitory Ly49 receptors suggests that c-*abl* is not required for the calibration of the NK cell repertoire (reviewed in Ref. 25). Moreover, c-*abl*-deficient A-LAKs demonstrated natural cytotoxicity and ADCC activities similar to their c-*abl*$^+$ counterparts, and c-*abl*$^{-/-}$ NK cells could eliminate MHC class I$^-$ tumor cells in vivo. Therefore, c-*abl*$^{-/-}$ NK cells appear normal in their development and in their effector functions.

The nature of the defect in c-*abl* mutant mice that causes lymphopenia and that predisposes these mice to infection remains elusive. Because c-*abl* expression is ubiquitous, effects of c-*abl* deficiency in *trans* may be difficult to dissociate from cell-intrinsic effects of the mutation. Previous studies using one strain of c-*abl* mutant mice showed variable defects in BM B cell development and peripheral lymphocyte function (11). These results suggested that the c-*abl* mutation could affect the function of T and B cells, although c-*abl* was not required for the development of these cells (9–12). Interestingly, the observed B and T cell defects could be transferred to normal mice by adult BM, but not by FL (11), strongly suggesting an in *trans* effect. Our results confirm that T and B cells are not strictly dependent on c-*abl* expression within fetal hemopoietic cells. Transfer of BM HSCs from adult c-*abl*$^{-/-}$ RAG2/γ_c chimeras to secondary RAG2/γ_c recipients also generated normal B, T, and NK cell development (our unpublished observations), arguing against a cell-intrinsic difference in c-*abl*$^{-/-}$ fetal vs adult HSCs. In addition, pre-B (B220$^+$IgM$^-$) cells from c-*abl*$^{-/-}$ RAG2/γ_c chimeras could generate mature B cells in vitro (our unpublished observations), in contrast to previous reports using freshly isolated pre-B cells from c-*abl*$^{-/-}$ mice (11). A major defect in NK cell functions as responsible for the susceptibility to infection observed in c-*abl* mutant mice also appears unlikely. RAG2/γ_c chimeras generated with c-*abl*-deficient FL cells show no increased mortality when housed in conventional animal facilities up to 8 mo postgraft. Taken together, these results argue against any important cell-intrinsic defects of the c-*abl* mutation for T, B, and NK cell development.

The complete absence of NK cells in RAG2/γ_c mice extends the RAG2 complementation system (16) to identify the genes responsible for and implicated in NK differentiation. Through the generation of somatic or hemopoietic chimeras in RAG2/γ_c mice, it should now be possible to establish the genetic map for the development of the NK cell lineage in vivo.

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