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Mice deficient in the *ALS2* gene exhibit lymphopenia and abnormal hematopietic function

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Abstract

One form of juvenile onset autosomal recessive amyotrophic lateral sclerosis (ALS2) has been linked to the dysfunction of the *ALS2* gene. The *ALS2* gene is expressed in lymphoblasts, however, whether *ALS2*-deficiency affects periphery blood is unclear. Here we report that *ALS2* knockout ($ALS2^{-/-}$) mice developed peripheral lymphopenia but had higher proportions of hematopoietic stem and progenitor cells in which the stem cell factor-induced cell proliferation was up-regulated. Our findings reveal a novel function of the *ALS2* gene in the lymphopoiesis and hematopoiesis, suggesting that the immune system is involved in the pathogenesis of ALS2.

Keywords

amyotrophic lateral sclerosis (ALS); ALS2; *ALS2* knockout mice; lymphopenia; hematopoietic stem and progenitor cells; cytokine-stimulated proliferation; stroma

1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease caused by a selective loss of lower and upper motor neurons in the central nerve system (CNS) (Cleveland and Rothstein, 2001). Recently, mutations in a second ALS-related gene (*ALS2*) were identified, which cause a rare recessive form of juvenile onset ALS (ALS2) (Hadano et al., 2001;Yang et al., 2001) adding onto the previously defined mutations in the copper/zinc superoxide dismutase 1-(SOD1) (Rosen, 1993). The fact that both *SOD1* and *ALS2* gene are expressed in a broad range of cell types in different tissues of various animal species led us to believe that mutations in these genes may affect systems other than the CNS. One of the systems warrants further investigation is the lymphohematopoietic system. A direct link between ALS and the lymphohematopoietic system comes from the clinical observation that ALS patients had marked lymphopenia with reductions in $CD2^+$ and $CD8^+$ T cells in comparison to age-matched normal controls (Provinciali et al., 1988). The “ALS”-like disorder seen in patients

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with HIV infection further suggests that reduced immunity with viral infection might be a key element in the pathogenesis of ALS (Verma and Berger, 2006;Sinha et al., 2004). Most recently, a significant decrease of white blood cells was observed in the $SOD1^{G93A}$ transgenic mouse model of ALS, further supporting the role of immunodeficiency in ALS (Kuzmenok et al., 2006). However, whether *ALS2*-deficiency affects immune system is not clear.

Previously, we along with others have developed a mouse model of ALS2 by generation of *ALS2* knockout ($ALS2^{-/-}$) mice (Cai et al., 2005;Hadano et al., 2006;Devon et al., 2006;Yamanaka et al., 2006). The resultant $ALS2^{-/-}$ mice exhibit deficits in motor activities but lack obvious motor neuron degeneration. Since the *ALS2* gene was also expressed in lymphoblasts from normal individuals (Yamanaka et al., 2003), the hematopoietic system could be affected in $ALS2^{-/-}$ mice. Here we examined lymphohematopoietic phenotypes in $ALS2^{-/-}$ mice. In comparison to wild-type ($ALS2^{+/+}$) littermate controls, $ALS2^{-/-}$ mice showed mild lymphopenia with enlarged $Lin^{-}Kit^{+}Sca1^{+}CD34^{-}$ HSC and $Lin^{-}Kit^{+}Sca1^{+}CD34^{+}$ hematopoietic progenitor cell pools. When cultured *in vitro* with stem cell factor (SCF), sorted $Lin^{-}CD117^{+}CD34^{+}$ and $Lin^{-}CD117^{+}CD34^{-}$ cells from $ALS2^{-/-}$ bone marrow (BM) expanded more than the same cells from wild type controls. BM cells from $ALS2^{-/-}$ mice also had reduced ability to form stromal feeder layer to support the growth of cobblestone colonies. Thus, Our findings reveal a novel function of the *ALS2* gene in the lymphopoiesis and hematopoiesis, suggesting that the immune system is involved in the pathogenesis of ALS2.

2. Materials and methods

2.1. Animals and antibodies

$ALS2^{-/-}$ mice were generated as previously described (Cai et al., 2005). All mice are on a mixed (129/SvJ × C57BL/6J) background. The mice were housed in a 12-hour light/dark cycle and fed regular diet *ad libitum*. The experimental protocols utilized in this paper are in accordance to the guidelines of the Institutional Animal Care and Use Committees of the National Institute of Child Health and Human Development. We used six pairs of young (3 months old) and four pairs of old (14 ± 1.89 months old, Mean ± SE) $ALS2^{-/-}$ and $ALS2^{+/+}$ littermate control mice in the current study. Monoclonal antibodies for murine CD3 (clone 145-2C11), CD4 (clone GK 1.5), CD8 (clone 53-6.72), CD11b (clone M1/70), CD19 (clone ID3), CD34 (clone RAM34), CD45R (B220, clone RA3-6B2), CD117 (CD117, clone 2B8), erythroid cells (clone Ter119), granulocytes (Gr1/Ly6-G, clone RB6-8C5), and stem cell antigen 1 (Sca1, clone E13-161) were all purchased from BD Biosciences (San Jose, CA). Antibodies were conjugated with fluorescein isothyocyanate (FITC), phycoerythrin (PE), phycoerythrin-Cyane 5 (PE-Cy5), or allophycocyanin (APC).

2.2. ALS2 expression in BM cells

BM cells were flushed from tibiae and femurs of $ALS2^{+/+}$ mice into Iscove's modified Dulbecco's medium (IMDM, ATCC, Manasses, VA). Erythrocytes were lysed by incubating with Gey's solution (130.68 mM NH_4Cl, 4.96 mM KCl, 0.82 mM Na_2HPO_4, 0.16 mM KH_2PO_4, 5.55 mM dextrose, 1.03 mM $MgCl_2$, 0.28 mM $MgSO_4$, 1.53 mM $CaCl_2$ and 13.39 mM $NaHCO_3$) for 10 minutes on ice. Residual cells were stained with a CD3-FITC + CD11b-Cychrome + CD45R-APC antibody cocktail and were sorted into $CD3^{+}$, $CD11b^{+}$, and $CD45R^{+}$ fractions. Cell sorting was carried out using a BD FACS Vantage SE with DiVa Cell Sorter (Becton Dickson, San Jose, CA), which was equipped with an Innova Argon 90 laser operating at 0.150 watt of visible line and a 0.50 watt Helium Neon laser operating at ~633 nm. Sorting was carried out at 35Psi and 71.1 kilohertz with less than 2.0% CV for each optical detector.

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Total RNA was isolated from each sorted cell fraction using the RNeasy Micro and QIAshredder kit (Qiagen, Valencia, CA). The SuperScript III One-Step RT-PCR System with Platinum Taq High Fidelity Kit (Invitrogen) was used to amplify *ALS2* mRNA covering exons 31 to 33 of *ALS2* gene with the forward primer *mALS2*-Ex31F: AGGATGCTTGCTTTGCATCT and the reverse primer *mALS2*-Ex33R: GCCTTCAAGGTGGTGAACAT. β-actin primers were used as controls. RT-PCR was performed at 1 cycle of 50°C for 30 min, 1 cycle of 94°C for 2 min, 20-21 cycles of amplification at 94°C for 30", 60-62°C for 30" and 72°C for 45", plus a final extension at 72°C for 10 min. The RT-PCR products for ALS2 were used as templates for a second round amplification using a pair of nested PCR primers of *ALS2* exons 31 to 33. The final PCR products were separated by electrophoresis through 2% agarose containing ethidium bromide.

2.3. Cell analysis and flow cytometry

Peripheral blood (PB) was obtained through retro-orbital sinus bleeding. One micro-tube of blood (~75 μl) was obtained from each animal for complete blood count (CBC) analyses using a Hemavet 950 analyzer (Drew Scientific Inc., Oxford, CT) whereas two more micro-tubes of blood was collected from each mouse for flow cytometry analyses. BM cells were flushed out from two tibiae and femurs of each animal into 2 mL of IMDM, filtered through a 90-μm nylon mesh (Small Parts Inc., Miami Lakes, FL), and counted by using a Vi-Cell XP Cell Viability Analyzer (Beckman-Coulter Corp, Hialeah, FL). Only viable cells were counted and used in calculations. Total BM cells per mouse were calculated based on the assumption that two tibiae and two femurs contain 25% of all BM cells in the body.

For flow cytometry analyses, blood and BM cells were incubated with Gey's solution twice of 10 minutes each at 4°C to lyse RBCs. Cells were then stained with pre-mixed antibody cocktails, and were analyzed using a BD-LSR II flow cytometer (Becton Dickinson, San Jose, CA). Each acquisition was stopped when 20,000 or 1,000,000 cells were collected, depending on the type of analysis.

2.4. SCF-stimulated cell proliferation in vitro

To study the effect of *ALS2* deletion on hematopoietic cell proliferation, we stained BM cells from *ALS2*$^{-/-}$ and wild-type mice with a pre-mixed antibody cocktail containing CD34-PE-Cy5 + Lineage (CD3, CD4, CD8, CD11b, CD19, Gr1, Ter119)-PE + CD117-APC, and sorted out $Lin^{-}CD117^{+}CD34^{+}$ and $Lin^{-}CD117^{+}CD34^{-}$ cell fractions using a BD FACS Vantage SE with DiVa Cell Sorter as described earlier. Sorted cells from *ALS2*$^{-/-}$ and wild-type mice were cultured in 12-well polystyrene flat bottom tissue culture plates (Corning Inc., Corning, NY 14831) at 3000 cells/well in 2 mL of Dulbecco's modified essential medium (DMEM, Cellgro Media tech, VA) supplemented with 15% heat-inactivated fetal bovine serum, 50 U/mL penicillin, 50 U/mL streptomycin, 2 μM L-glutamine, 3 ng/mL IL-3, and 5 ng/mL IL-6. Cells were cultured in duplicate or triplicate wells at 37°C with 5% $CO_2$with or without the addition of 25 ng/mL SCF. At the end of the 7-day culture, cells were harvested and counted under a light microscope to calculate cell expansion defined as the ratio between the number of cells recovered and the number of cells used to initiate culture.

2.5. Data Analysis

Data collected from different assays were statistically analyzed using the JMP statistical discovery software with least square models (SAS et al., 1998). Results shown in Tables or Figures were means and standard errors, while statistical significance was declared at $P<0.05$ and $P<0.01$ levels respectively.

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3. Results

3.1. ALS2 is widely expressed in BM cells of all hematopoietic lineages

A previous experiment reports that *ALS2* is expressed in the lymphoblasts (Yamanaka et al., 2003). However, whether the *ALS2* gene is expressed in BM cells of all hematopoietic lineages is unclear. In our initial experiment, we examined the expression of *ALS2* mRNA in sorted $CD3^+$ T cells, $CD45R^+$ B cells, and $CD11b^+$ myeloid cells as well as in unfractionated whole BM cells. Data from RT-PCR analyses indicated that *ALS2* gene is ubiquitously expressed in T and B cells of the lymphoid cell lineage as well as in cells of the myeloid lineage, despite that the signal was slightly weaker in myeloid cells (Fig. 1).

3.2. Circulating leukocyte number is reduced in ALS2$^{-/-}$ mice

To investigate whether the blood cells are affected by loss of the *ALS2* gene, we measured the peripheral blood cellular composition in *ALS2*$^{+/+}$ and *ALS2*$^{-/-}$ mice at 3 and 14 months of age. Circulating white blood cells (WBCs) reduced significantly ($P<0.01$) with age in *ALS2*$^{+/+}$ and *ALS2*$^{-/-}$ mice, whereas *ALS2*$^{-/-}$ mice had significantly fewer ($P<0.05$) WBCs than *ALS2*$^{+/+}$ littermates at both young and older ages (Table 1). The reduction in WBC concentration in *ALS2*$^{-/-}$ mice was caused by a decline the concentration of lymphocytes in these mice compared to the wild-type controls ($P<0.08$). In contrast, blood concentrations of neutrophils, monocytes, basophils and eosinophils showed no obvious difference between *ALS2*$^{+/+}$ and *ALS2*$^{-/-}$ mice (Table 1). In addition, the red blood cells (RBC) and platelet concentrations did not change in *ALS2*$^{-/-}$ mice either.

In partitioning peripheral blood leukocytes into different subsets, we found that proportions of $CD4^+$, $CD8^+$, and $CD11b^+$ were not different between *ALS2*$^{+/+}$ and *ALS2*$^{-/-}$ mice, despite that both $CD4^+$ ($P<0.01$) and $CD8^+$ ($P<0.05$) cell proportions were significantly reduced with age (Table 2). Older *ALS2*$^{-/-}$ mice had lower proportions of $CD45R^+$ B cells and higher proportions of $Ter119^+$ erythrocytes than *ALS2*$^{+/+}$ mice, but these differences did not reach the level of significance (Table 2). In comparison to *ALS2*$^{+/+}$ controls, *ALS2*$^{-/-}$ mice seemed to have slightly higher proportion of CD4 cells but lower proportions of CD8 T cells and CD45R B cells in both age groups (Table 2), although these differences were not statistically significant. Thus, reduction in lymphocytes in *ALS2*$^{-/-}$ mice might associate with further reduction of cytotoxic T cells (CD8) and the antibody-producing B cells (CD45R).

3.3. Age-associated upregulation in hematopoietic cell proliferation in ALS2$^{-/-}$ mice

The reduced number of mature leukocytes in *ALS2*$^{-/-}$ mice could be contributed by a reduced number of hematopoietic precursors in the BM. To test this hypothesis, we stained BM cells using previously established marker combinations for mature and immature progenitor cells. We found that the proportion of $CD4^+$ cells was actually higher ($P<0.01$) in older *ALS2*$^{-/-}$ than in age-matched *ALS2*$^{+/+}$ mice (Table 3). Proportions of $CD8^+$, $CD11b^+$, and $CD45R^+$ cells were all insignificantly lower in *ALS2*$^{-/-}$ than in *ALS2*$^{+/+}$ mice, consistent with observations from the blood (Table 2), whereas proportion of $Ter119^+$ erythrocytes was significantly ($P<0.05$) higher in *ALS2*$^{-/-}$ mice than in *ALS2*$^{+/+}$ controls (Table 3). At young age, *ALS2*$^{-/-}$ mice had similar proportions of $Lin^-Sca1^+CD117^+$, $Lin^-Sca1^+CD117^+CD34^+$, and $Lin^-Sca1^+CD117^+CD34^-$ cells in the BM as *ALS2*$^{+/+}$ mice, whereas at old age *ALS2*$^{-/-}$ mice had significantly higher ($P<0.01$) proportions of all these cell populations than those in *ALS2*$^{+/+}$ mice (Fig. 2).

The higher proportions of BM hematopopietic progenitor and stem cells in older *ALS2*$^{-/-}$ mice suggested that the deletion of *ALS2* gene might cause cells to be more responsive to cytokine stimulation-induced cell proliferation. To test this hypothesis, we sorted $Lin^-CD117^+CD34^+$ and $Lin^-CD117^+CD34^-$ cells from the BM of *ALS2*$^{-/-}$ and *ALS2*$^{+/+}$ mice, and cultured them

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in vitro for 7 days in the presence of IL-3 and IL-6 with and without 25 ng/mL SCF. We found that the $Lin^{-}CD117^{+}CD34^{+}$ cells from *ALS2*$^{-/-}$ mice expanded significantly more than the cells from *ALS2*$^{+/+}$ mice (*ALS2*$^{-/-}$: 507 ± 66 folds *vs.* *ALS2*$^{+/+}$: 297 ± 58 folds; $p < 0.05$; Fig. 3). Moreover, the $Lin^{-}CD117^{+}CD34^{-}$ cells from *ALS2*$^{-/-}$ mice also expanded more (357 ± 66 folds) than the same cells from *ALS2*$^{+/+}$ mice (286 ± 93 folds), although this difference was not statistically significant. Taken together, both $Lin^{-}CD117^{+}CD34^{+}$ and $Lin^{-}CD117^{+}CD34^{-}$ cells from *ALS2*$^{-/-}$ mice were more responsive to SCF stimulation than the same cells from *ALS2*$^{+/+}$ controls. This response, along with the observed peripheral lymphocytopenia, suggest that *ALS2* is involved in the regulation of hematopoietic lineage commitment toward T and B cells. Deficiency of *ALS2* gene product causes deficiency in lymphocyte commitment which, as an compensatory effect, causes up regulation in the proliferation of hematopoietic stem and progenitor cells.

4. Discussion

In this report, we showed that loss of *ALS2* gene product resulted in peripheral lymphopenia with reduced proportions of CD8 T cells and CD45R B cells. Proportions of hematopoietic stem and progenitor cells were much higher in *ALS2*-deficient mice, probably because these cells are more sensitive to cytokine-stimulated cell proliferation. Our observations, along with these previous reports (Provinciali et al., 1988; Kuzmenok et al., 2006), suggest a causal link between ALS and lymphopenia.

It will be interesting to know how the *ALS2* gene is involved in lymphohematopoiesis and immune function. Based on sequence homology, alsin encoded by the full-length *ALS2* gene contains three putative guanine-nucleotide-exchange factor (GEF) like domains: an amino-terminal regulator of chromatin condensation (RCC1) like domain (RLD), a middle Dbl homology (DH)/pleckstrin homology (PH) like domain, and a carboxyl-terminal vacuolar protein sorting 9 (VPS9) like domain, which are homologous to GEFs of Ran, Rho, and Rab GTPase, respectively (Hadano et al., 2001;Yang et al., 2001). Previous studies have shown that the VPS9 domain of alsin in conjunction with its upstream membrane occupation and recognition nexus motifs can specifically activate the Rab5 subfamily GTPases (Otomo et al., 2003;Topp et al., 2004), and the DH/PH domain of alsin is believed to facilitate the activation of members of Rho GTPases (Topp et al., 2004). The role of Rho GTPases in the regulation of hematopoiesis has been well studied in the past. Both Rac1 and Rac2, the hematopoietic-specific Rho family GTPases, play important roles in neutrophil function and host defense (Roberts et al., 1999), phagocyte immunodeficiency (Williams et al., 2000), and hematopoietic cell migration, localization and interaction with microenvironment (Jansen et al., 2005;Cancelas et al., 2005;Gottig et al., 2006). Moreover, Cdc42GAP-deficient mice are anemic with significant declines in BM cells and the numbers of erythroid blast-forming unit (BFU-E) and colony-forming unit (CFU-E) (Wang et al., 2006). Cdc42gap is a negative regulator of Rho family GTPase, Cdc42. We speculate that deficiency in alsin may alter the activities of Rho family GTPases in BM stem and progenitor cells and therefore affects lymphohematopoiesis. However, the mechanism of alsin in lymphohematopoiesis remains to be defined.

Similar to *ALS2*$^{-/-}$ mice, Trp53 and IL-2-deficient mice also have higher numbers of $Lin^{-}Sca1^{+}CD117^{+}CD34^{-}$ hematopoietic stem cells (HSCs) in the BM (Chen et al., 2002;TeKippe et al., 2003). Interestingly, Trp53$^{-/-}$ and IL-2$^{-/-}$ mice display significantly different HSC function. When tested in competitive repopulation experiments *in vivo*, Trp53$^{-/-}$ mice expressed very high HSC engraftment activity while IL-2$^{-/-}$ mice expressed very low HSC engraftment (Chen et al., 2002; TeKippe et al., 2003). It will be nice to examine the HSC engraftment activity in *ALS2*$^{-/-}$ mice. However, it will be difficult to interpret the engraftment assay using HSCs derived from *ALS2*$^{-/-}$ mice, since a mixed genetic background

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of *ALS2*$^{-/-}$ mice in which too many minor-histocompartibility antigen disparities will dramatically interfere with the outcome of HSC engraftment. A congenic line of *ALS2*$^{-/-}$ mice in C57BL6 background is under development to address this issue.

In summary, although the mechanism of abnormal lymphohematopoiesis and stromal function in *ALS2*$^{-/-}$ mice remains to be addressed, we are the first to reveal a novel function of alsin in the development of blood cells, suggesting that the immune system is involved in the pathogenesis of ALS2.

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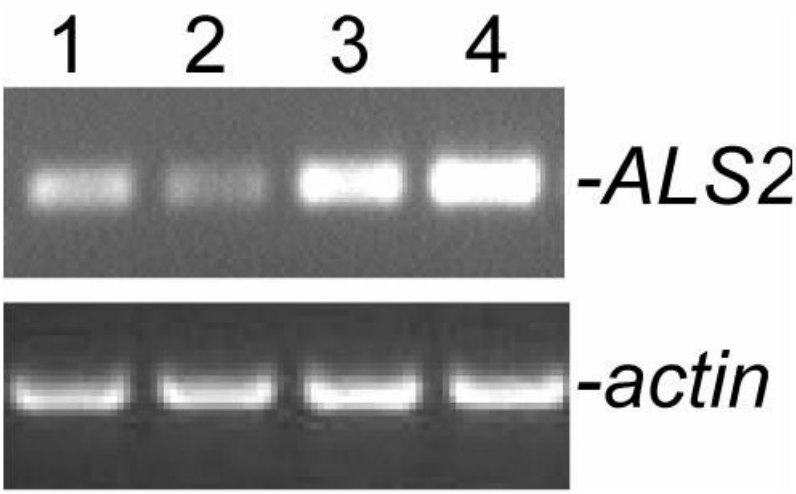

Fig. 1.

Total RNA was extracted from sorted CD45R$^+$(lane 1), CD11b$^+$ (lane 2), CD3$^+$ (lane 3) and unfractionated whole BM cells (WBM, lane 4) from young *ALS2*$^{+/+}$ mice and subjected to reverse transcriptase-PCR (RT-PCR) analyses of *ALS2* (upper panel) and β-*actin* gene expression (lower panel).

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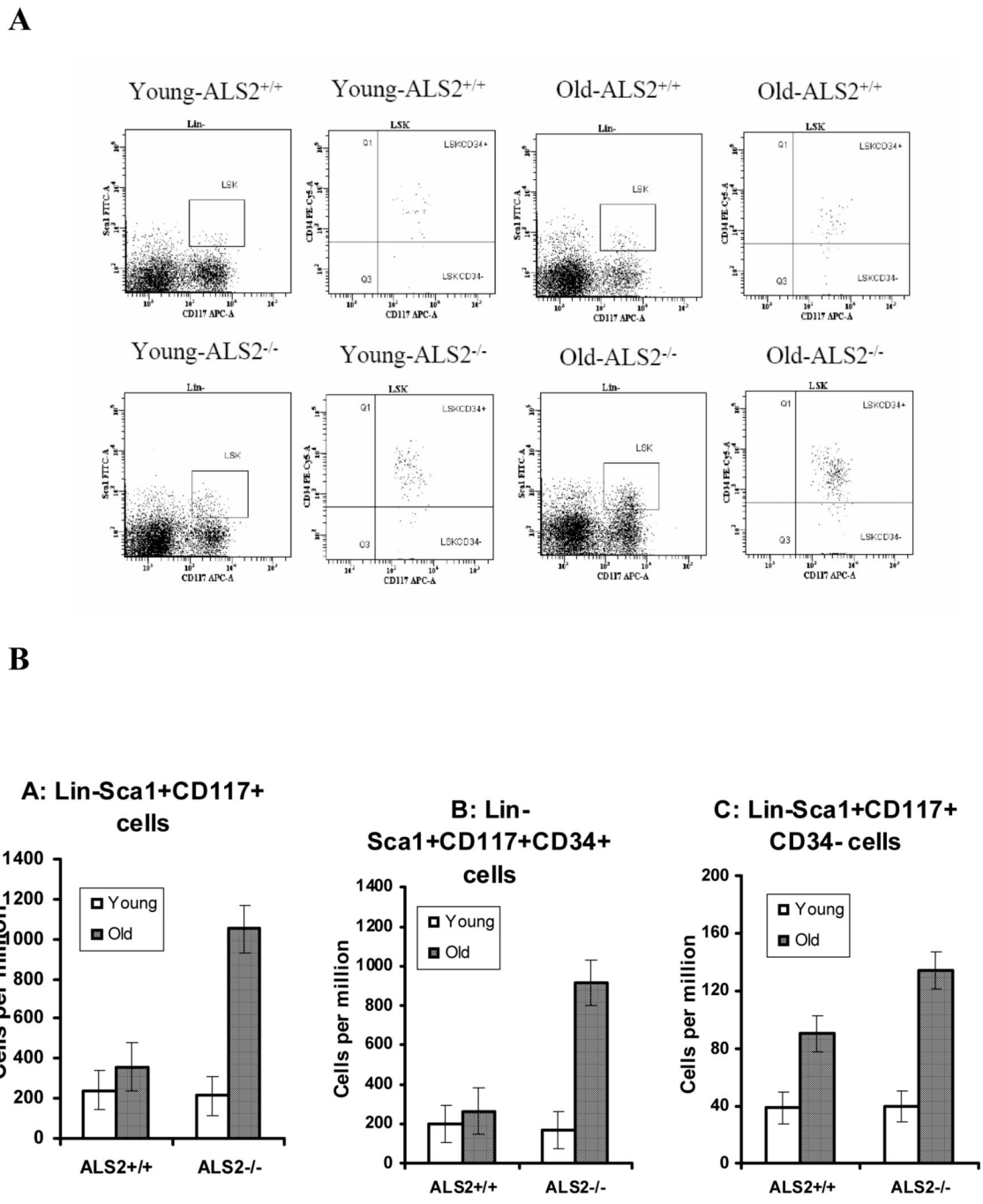

Fig. 2.

BM cells extracted from young (3 months) and old (14 months) mice were stained with Sca1-FITC + Lin (CD3, CD4, CD8, CD11b, CD45R, Gr1, Ter119)-PE + CD34-PE-Cy5 + CD117-APC and analyzed by a LSR II flow cytometry. Lin- cells were gated first to display the Sca1CD117 double positive cell population as the LSK cells, which were then gated to display CD34 expression. Proportions of $Lin^{-}Sca1^{+}CD117^{+}$ and $Lin^{-}Sca1^{+}CD117^{+}CD34^{+}$ cells increased significantly with age in $ALS2^{-/-}$ but not in $ALS2^{+/+}$ mice while proportion of $Lin^{-}Sca1^{+}CD117^{+}CD34^{-}$ cells increased with age in both $ALS2^{-/-}$ and $ALS2^{+/+}$ mice. Data shown as representative dot plots (**A**) as well as least square means with standard error bars from 4 mice analyzed for each genotype/age group (**B**).

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Fig. 3.

Sorted $Lin^{-}CD117^{+}CD34^{+}$ and $Lin^{-}CD117^{+}CD34^{-}$ BM cells from young *ALS2*$^{+/+}$ and *ALS2*$^{-/-}$ mice were cultured in DMEM in 12-well polystyrene tissue culture plates at 3000 cells/well in the presence of 3 ng/mL IL-3 and 5 ng/mL IL-6 with or without 25 ng/mL SCF. Cells were cultured at 37°C with 5% $CO_2$for 7 days. Harvested cells were counted under a light microscope to calculate fold of cell expansion defined as the ratio between the number of cells recovered and the number of cells used to initiate culture. Data presented as means with standard error bars from duplicate or triplicate culture wells for each cell type.

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Table 1

Complete blood count

Genotype	Young		Old		Stat
	ALS2$^{+/+}$	*ALS2*$^{-/-}$	*ALS2*$^{+/+}$	*ALS2*$^{-/-}$	
WBC (10^6/mL)	10.0 ± 0.8	6.7 ± 0.8	6.1 ± 1.1	4.8 ± 1.0	A, g
Lymphocytes (10^6/mL)	6.8 ± 0.8	4.4 ± 0.8	3.1 ± 1.2	2.0 ± 1.0	A
Neutrophils (10^6/mL)	2.9 ± 0.5	1.9 ± 0.5	2.6 ± 0.8	2.4 ± 0.8	NS
Monocytes (10^6/mL)	0.225±0.085	0.270±0.085	0.357±0.120	0.315±0.104	NS
Basophiles (10^6/mL)	0.030±0.017	0.015±0.020	0.030±0.024	0.063±0.020	NS
Eosinophiles (10^6/mL)	0.007±0.003	0.002±0.003	0.010±0.004	0.008±0.003	NS
RBC (10^9/mL)	9.7 ± 0.4	9.6 ± 0.4	9.5 ± 0.5	8.6 ± 0.5	NS
Platelets (10^6/mL)	834 ± 84	711 ± 84	1041 ± 119	919 ± 103	NS

Complete blood counts were performed for young (3 months) and older (14 months) ALS2 wild type (*ALS2*$^{+/+}$) and ALS2 knockout (*ALS2*$^{-/-}$) mice. Data shown are least square means ± standard errors of 6 mice from each of the two young groups and of 4 mice from each of the two old groups after variance analyses. Statistics: A: significant age effect ($P<0.01$); g: significant genotype effect ($P<0.05$); NS: no significance.

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Table 2

Cellular composition in peripheral blood

Genotype	CD4%	CD8%	CD11b%	CD45R%	Ter119%
Young *ALS2*$^{+/+}$	13.6 ± 2.5	10.1 ± 1.8	23.6 ± 5.9	34.4 ± 4.7	13.1 ± 7.5
Young *ALS2*$^{-/-}$	15.0 ± 2.5	9.9 ± 1.8	23.4 ± 5.9	32.7 ± 4.7	13.8 ± 7.5
Old *ALS2*$^{+/+}$	5.1 ± 3.5	4.6 ± 2.5	20.2 ± 7.2	24.5 ± 5.8	22.0 ± 9.2
Old *ALS2*$^{-/-}$	5.4 ± 3.0	3.9 ± 2.2	30.8 ± 7.2	19.2 ± 5.8	35.7 ± 9.2
Statistics	A	a	NS	a	NS

Peripheral blood from young (3 months) and older (14 months) *ALS2* wild type (*ALS2*$^{+/+}$) and *ALS2* knockout (*ALS2*$^{-/-}$) mice were analyzed by flow cytometry. Data shown are least square means ± standard errors of 6 mice from each of the two young groups and 4 mice from each of the two old groups after variance analyses. Statistic significance: A: significant age effect ($P<0.01$); a: significant age effect ($P<0.05$); NS: no significance.

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Table 3

Cellular composition in BM

Genotype	CD4%	CD8%	CD11b%	CD45R%	Ter119%
Young *ALS2*$^{+/+}$	1.6 ± 0.1	1.3 ± 0.2	46.9 ± 4.2	19.7 ± 4.6	20.1 ± 3.2
Young *ALS2*$^{-/-}$	1.4 ± 0.1	1.1 ± 0.2	37.4 ± 4.6	18.9 ± 5.1	29.4 ± 3.5
Old *ALS2*$^{+/+}$	1.9 ± 0.2	1.4 ± 0.3	41.1 ± 5.1	24.0 ± 5.7	25.6 ± 3.9
Old *ALS2*$^{-/-}$	2.9 ± 0.2	1.2 ± 0.3	37.7 ± 5.1	20.2 ± 5.7	36.6 ± 3.9
Statistics	A, g, A*G	NS	NS	NS	g

BM cells from young (3 months) and old (14 months) *ALS2* wild type (*ALS2*$^{+/+}$) and *ALS2* knockout (*ALS2*$^{-/-}$) mice were analyzed by flow cytometry. Data shown are least square means ± standard errors of 6 mice from each of the two young groups and 4 mice from each of the two old groups after variance analyses. Statistic significance: A: significant age effect (P<0.01); g: significant genotype effect (P<0.05); A*G: significant age*genotype interaction (P<0.05); NS: no significance.