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PLENARY PAPER

Loss of *FancC* Function Results in Decreased Hematopoietic Stem Cell Repopulating Ability

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Fanconi anemia (FA) is a complex genetic disorder characterized by progressive bone marrow (BM) aplasia, chromosomal instability, and acquisition of malignancies, particularly myeloid leukemia. We used a murine model containing a disruption of the murine homologue of *FANCC* (*FancC*) to evaluate short- and long-term multilineage repopulating ability of *FancC* −/− cells in vivo. Competitive repopulation assays were conducted where "test" *FancC* −/− or *FancC* +/+ BM cells (expressing CD45.2) were cotransplanted with congenic competitor cells (expressing CD45.1) into irradiated mice. In two independent experiments, we determined that *FancC* −/− BM cells have a profound decrease in short-term, as well as long-term, multilineage repopulating ability. To determine quantitatively the relative production of progeny cells by each test cell population, we calculated test cell contribution to chimerism as compared with 1 × 10^5 competitor cells. We determined that *FancC* −/− cells have a 7-fold to 12-fold decrease in repopulating ability compared with *FancC* +/+ cells. These data indicate that loss of *FancC* function results in reduced in vivo repopulating ability of pluripotential hematopoietic stem cells, which may play a role in the development of the BM failure in FA patients. This model system provides a powerful tool for evaluation of experimental therapeutics on hematopoietic stem cell function.

FANCONI ANEMIA (FA) is a complex, heterogeneous genetic disorder characterized clinically by a predisposition to congenital anomalies, a progressive bone marrow (BM) aplasia, and the development of malignancies.[1-3] The diagnosis of FA is established by a characteristic hypersensitivity of FA cells to bifunctional alkylating agents such as mitomycin C (MMC) or diepoxybutane (DEB).[3,4] This cellular hypersensitivity is manifested by induction of chromosome breaks, a G_2/M cell cycle arrest, and cell death (reviewed in D'Andrea and Grompe[5] and Auerbach and Verlander[6]). The hypersensitivity of FA cells to MMC has enabled the use of somatic cell fusion studies to identify eight complementation groups (A to H), inferring the existence of eight genes.[7] Four of the eight genes have been mapped to different chromosomal loci (*FANCA*, *FANCC*, *FANCD*, *FANCG*), and the *FANCA*, *FANCC*, and *FANCG* genes have been cloned.[8-12]

Many patients with FA have non–life-threatening congenital abnormalities particularly of the skeletal, genitourinary, and central nervous systems.[13,14] However, the major causes of morbidity and mortality of FA relate to dysfunction of the hematopoietic system. Most patients die of BM failure (80%) or develop malignancies, particularly myeloid leukemia. The mean age of onset of BM failure occurs at 8 years, and the mean survival is 19 years.[2] Data from many laboratories indicate that there is a reduction in the frequency of lineage restricted erythroid (burst-forming unit-erythroid [BFU-E]) and granulocyte macrophage (colony-forming unit–granulocyte-macrophage [CFU-GM]) progenitors from BM aspirates of asymptomatic,[15] as well as pancytopenic FA patients.[16,17] These studies have also demonstrated a decrease in colony size, possibly reflecting a reduction in proliferation of lineage restricted progenitors. Together these data infer that loss of function of an FA protein results in a qualitative or quantitative alteration in the progenitor compartment. In addition, the development of progressive BM failure in most FA patients suggests that loss of FA gene function results in injury to the stem cell, as well as the progenitor cell compartment.

Recently, two murine models containing a disruption of the murine homologue of *FANCC* (*FancC*) were developed to facilitate functional studies of primary cells in vitro and in vivo. Chen et al[18] created a disruption in exon 8 of *FancC*, while

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Whitney et al[19] used homologous recombination to create a disruption in exon 9. In both lines of mice, spontaneous chromosomal breaks in splenic lymphocytes were observed, as well as an increase in chromosomal breaks in response to bifunctional alkylating agents. In addition, a number of abnormalities in the progenitor cell compartment were identified in both murine models, including the hyperresponsiveness of *FancC* −/− progenitors to MMC[20,21] and a predisposition of *FancC* −/− progenitors to undergo apoptosis in response to inhibitory cytokines.[20,22] The murine line developed by Whitney et al[19] develops an age-dependent decrease in the number of hematopoietic progenitors cultured from the BM, while *FancC* −/− mice developed by Chen et al[18] do not acquire these functional abnormalities, suggesting a somewhat milder phenotype. Cumulatively, the similarities between in vitro BM assays in FA patients and those in *FancC* −/− mice suggest that these mice will be a good model system to evaluate the role of *FancC* on short- and long-term in vivo stem cell repopulating ability.

A major advantage of using murine models to study genetic diseases affecting the hematopoietic system is the ability to evaluate hematopoietic stem cell function by repopulation in vivo, the only universally accepted assay for these cells. These transplantation studies can be accomplished using well-established, competitive repopulation assays, which allow highly accurate, quantitative determinations of stem cell activity.[23-30] Congenic mouse strains that differ only in the expression of genetically distinguishable isoenzymes, such as the CD45.1/CD45.2 antigens, allow identification of progeny cells from two different stem cell populations with high sensitivity. Coinjection of ''competitor'' cells of one isotype and ''test'' cells with a distinct isotype into irradiated recipients provides a means to identify stem cells that have different capacities to compete for repopulation.

We used *FancC* −/− mice to address two questions related to the role of *FancC* in regulating hematopoietic stem cell control. First, we used the competitive repopulation assay to determine whether *FancC* is required for normal repopulating ability of short- and long-term reconstituting hematopoietic stem cells. Second, because the hematopoietic system is hierarchical in nature and has multiple compartments, we evaluated whether *FancC* is required to regulate the growth of specific populations of cells. We demonstrate that loss of *FancC* results in a profound impairment in short- and long-term repopulating stem cells. These data have significance for understanding the basic pathogenesis of the disease and in providing an approach to test experimental therapies.

MATERIALS AND METHODS

Mice. *FancC* −/− and *FancC* +/+ mice (C57Bl/6 × SV129) were backcrossed into a C57Bl/6 strain (CD45.2$^+$). Congenic C57Bl/6 (CD45.2$^+$) and B6.SJL-PtrcaPep3b/BoyJ (B6.BoyJ) mice (CD45.1$^+$) were purchased from Jackson Laboratories (Bar Harbor, ME) and maintained in our animal facility.

Harvesting BM samples. BM cells were flushed from the tibias and femurs of 6- to 8-week-old *FancC* −/− and *FancC* +/+ littermates and B6.BoyJ mice using Iscove's Modified Dulbecco's Media (IMDM) (GIBCO-BRL, Gaithersburg, MD) containing 5% fetal calf serum (FCS) (Hyclone Laboratories, Logan, UT). Low-density mononuclear cells (LDMNC) were prepared by centrifugation on ficoll-hypaque (density, 1.119; Sigma, St Louis, MO). B6.BoyJ LDMNC were used as competitor cells for both competitive repopulation experiments. *FancC* −/− and *FancC* +/+ LDMNC were further purified by fluorescence cytometry for Sca1$^+$ lin$^{-/dim}$ cells to be used as test cells.

Purification of Sca1$^+$ lin$^{-/dim}$ cells. BM LDMNC from littermates of each genotype were pooled before cell sorting. The cells were resuspended in cold phosphate-buffered saline (PBS) 0.1% bovine serum albumin (BSA) at a concentration of 1 to 2 × 10^8 cells/mL. The cells were stained for 20 minutes at 4°C with the following antibodies: Sca1-phycoerythrin (PE), CD4-fluorescein isothiocyanate (FITC), CD8-FITC, B220-FITC, Mac1-FITC, Gr1-FITC, and Ter119-FITC. All antibodies were purchased from PharMingen (San Diego, CA). Cell samples were washed twice and resuspended at a concentration of 5 to 10 × 10^6 cells/mL cold PBS 0.1% BSA. The cells were sorted for Sca1$^+$ lin$^{-/dim}$ cells using a Becton Dickinson (San Jose, CA) FACSTAR sorter using identical sorting gates. This selection enriches for immature hematopoietic progenitor and stem cells and excludes all differentiated cells.

Competitive repopulation experiments. Ninety 8- to 10-week-old female C57Bl/6 mice (experiment 1) or B6.BoyJ mice (experiment 2) were lethally irradiated (1,100 cGy split dose) before transplantation as previously described.[31,32] Limiting dilutions of Sca1$^+$ lin$^{-/dim}$ cells (0 to 5,000 experiment 1 and 0 to 10,000 experiment 2) from *FancC* −/− and *FancC* +/+ mice were mixed with a constant number of B6.BoyJ low-density competitor cells (5 × 10^5). Each cell mixture was resuspended in 0.5 mL of IMDM 2% FCS and injected into the tail vein of six lethally irradiated recipients. Some mice (n = 4) were irradiated, but did not receive exogenous cells to be certain that the irradiation dose was lethal and to control for any residual contribution of the host's hematopoietic system. Finally, to control for any potential late contribution of endogenous hematopoiesis from the irradiated recipient animals, some mice (n = 6) were transplanted with competitor cells only.

Chimerism analysis by fluorescence cytometry. Tail-vein blood samples (100 μL) were obtained monthly posttransplantation for analysis of chimerism and every other month for multilineage analysis. Peripheral blood cells were incubated in red blood cell (RBC) lysis buffer (0.16 mol/L NH_4Cl, 0.1 mol/L $KHCO_3$, 0.1 mmol/L EDTA) for 5 minutes at 4°C. The cells were washed twice, resuspended in PBS 0.1% BSA, and aliquoted into seven individual tubes for antibody staining. Each sample was stained with CD45.1-FITC (B6.BoyJ strain) and CD45.2-FITC (C57Bl/6 strain) plus four individual lineage markers conjugated to PE at 4°C for 20 minutes. Samples were washed twice and resuspended in PBS 0.1% BSA before analysis by fluorescence cytometry. Three mixtures of C57Bl/6 and B6.BoyJ cells were stained with CD45.1-FITC and CD45.2-FITC individually as controls to assist with appropriate gate settings and to assure that no errors occurred during antibody staining. A total of 5,000 events were collected from each sample. All data were analyzed using CELLQuest software (Becton Dickinson). Instrument settings and gates used to analyze data were identical from month to month and between genotypes. An unpaired Student's *t*-test was used to determine whether significant differences existed in chimerism between genotypes.

Repopulating unit calculation. Relative repopulating ability of the donor cells compared with the competitor cell population was determined using a repopulating unit (RU) calculation described previously by Harrison et al.[27,28] This calculation allows quantitative comparison of repopulating ability between different donor cell populations, ie, *FancC* −/− and *FancC* +/+. We calculated RU for mice transplanted with the highest dose of Sca1$^+$ lin$^{-/dim}$ cells for both experiments. Because a constant number of competitors (5 × 10^5) were used for all experiments, the following equation was used to compute RU: RU = 5 × Measured Donor Chimerism/(100 − Measured Donor Chimerism). Differences in RU between groups were compared using an unpaired Student's *t*-test.

RESULTS

Chimerism detection using fluorescence cytometry. Preliminary studies were conducted to evaluate the reliability of fluorescence cytometry to detect CD45.1 and CD45.2 antigen positive cells over a range of C57Bl/6 and B6.BoyJ cell mixtures. Defined numbers of nucleated peripheral blood cells of each respective strain were mixed and stained with antibodies to either CD45.1-FITC or CD45.2-FITC. Addition of the measured CD45.1 and CD45.2 percentages was used as an internal control to verify that the sum of the two percentages approximated 100%. The results from these studies are shown in Fig 1. The experimental result obtained from measuring CD45.1 or CD45.2 was consistently within 3% of the predicted value. These results support the use of fluorescence cytometry as a sensitive and accurate methodology to analyze donor cell contribution in animals transplanted with cells expressing these two isoantigens over a wide range of chimerism.

FancC −/− hematopoietic stem cells have decreased short- and long-term repopulating ability. A schematic of the competitive repopulation assay is illustrated in Fig 2. BM cells from 6- to 8-week-old *FancC* −/− and *FancC* +/+ littermates were isolated, fractionated using ficoll-hypaque separation, and sorted by fluorescence-activated cell sorting (FACS) to obtain $Sca1^+$ $lin^{-/dim}$ cells. The percentage of nucleated BM cells from both *FancC* −/− and *FancC* +/+ mice that were $Sca1^+$ $lin^{-/dim}$ ranged from 0.4% to 0.5%. The absolute number of nucleated and $Sca1^+$ $lin^{-/dim}$ cells per femur were similar in both *FancC* +/+ and *FancC* −/− cells, consistent with previously published studies[20] (data not shown). These cells were mixed with 5×10^5 competitor cells and injected into irradiated recipient mice.

Peripheral blood samples were collected from recipient mice monthly after transplantation, and aliquots of cells from each specimen were stained with CD45.1-FITC and CD45.2-FITC, respectively. Representative histograms of chimeric peripheral blood cell specimens from mice transplanted with 5,000 $Sca1^+$ $lin^{-/dim}$ *FancC* +/+ or *FancC* −/− (express CD45.2) and 500,000 low-density mononuclear competitor cells (express CD45.1) 6 months after transplantation are shown in Fig 3. The CD45.2 chimerism measured from the mouse transplanted with *FancC* −/− cells was only 18%, while the CD45.2 chimerism in the recipient transplanted with *FancC* +/+ cells was 71%.

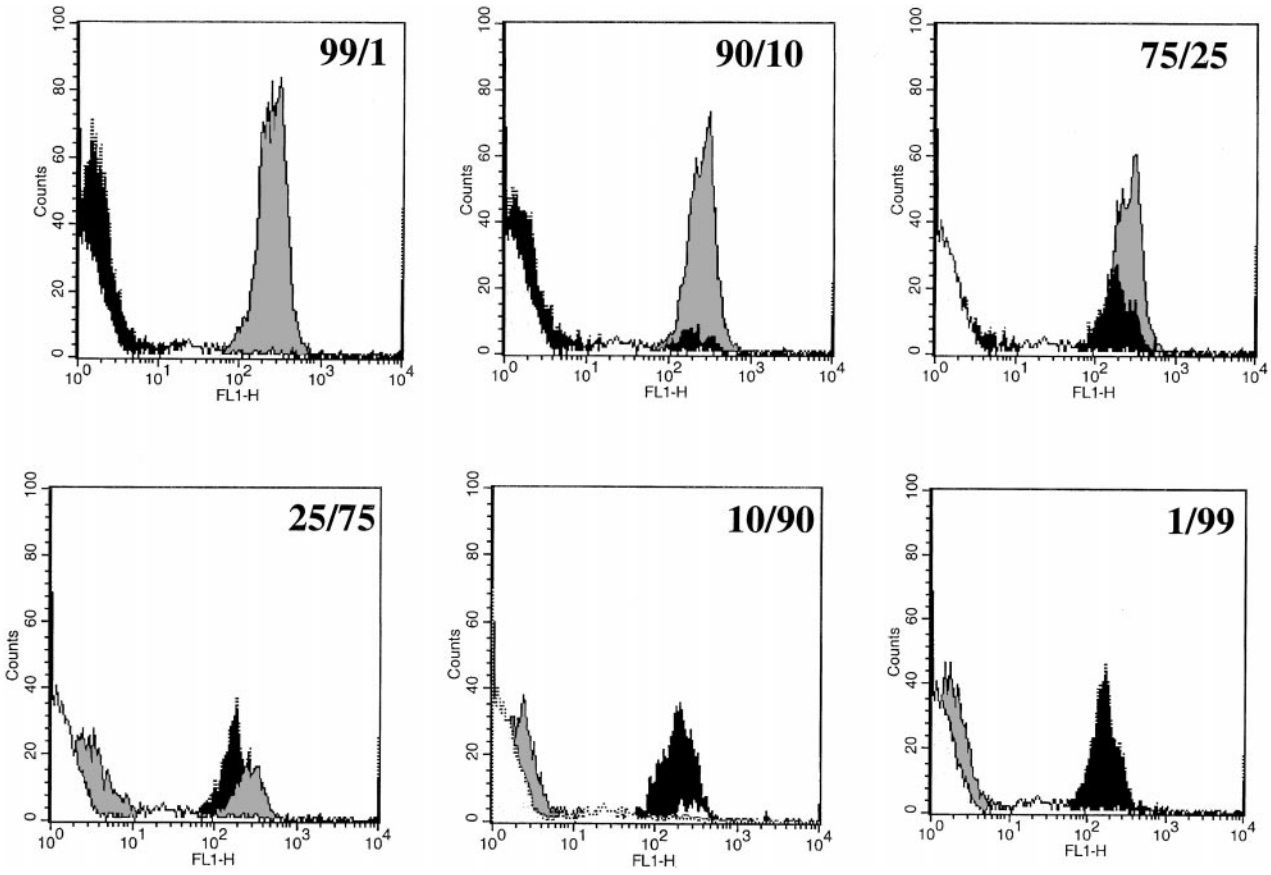

Fig 1. Detection of test cell chimerism using fluorescence cytometry. Venous peripheral blood cells were isolated from the tail veins of C57Bl/6 ($CD45.2^+$) and B6.BoyJ ($CD45.1^+$) mice. Defined mixtures of nucleated blood cells from each mouse were stained with antibodies to CD45.1 (▒), CD45.2 (■), or a nonspecific isotype control (□) and analyzed using fluorescence cytometry. Each histogram represents the staining profile of a single cell mixture. The white peak is the isotype control. The gray and black peaks represent CD45.1 and CD45.2 positive cells, respectively. The numbers in the upper right corner of each histogram show the expected percentages of CD45.1/CD45.2 for each histogram. CD45.1 and CD45.2 chimerism measured using fluorescence cytometry were consistently within 3% of the predicted value over a wide range of cell mixtures.

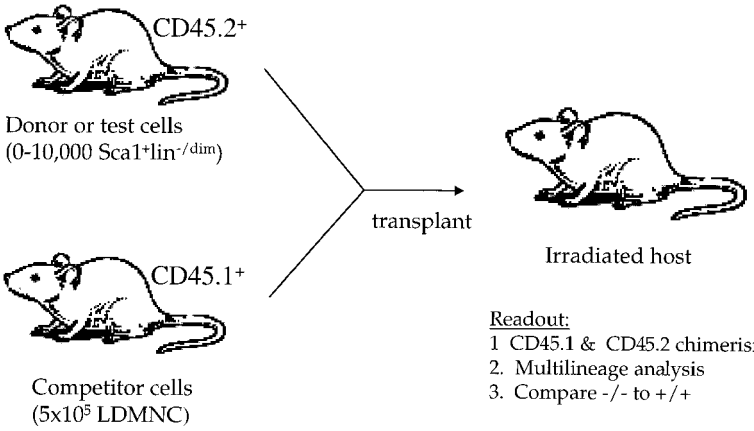

Fig 2. Schematic of competitive repopulation assay. Test cells from the BM of *FancC −/−* and *FancC +/+* littermates were isolated and enriched for Sca1^{+}lin$^{-/dim}$ cells. Each respective test cell population was cotransplanted into irradiated recipient mice with competitor cells from B6.BoyJ mice that were genetically identical to C57Bl/6 mice with the exception of expression of a different CD45 isoantigen.

As expected, in each case the sum of CD45.1 chimerism (representing competitor cell proliferation) and CD45.2 chimerism (representing test cell proliferation) approximated 100% ± 3%.

Short-term repopulating ability is characteristically measured 1 month after transplantation and long-term repopulating ability is established by 4 to 6 months after transplantation in the murine system.[26-28] The CD45.2 chimerism of all transplanted mice from a representative experiment at 1, 3, and 6 months after transplantation is shown in Fig 4. As expected, all animals irradiated, but not transplanted with exogenous cells, died in 14 days, and recipient animals (CD45.2^{+}) transplanted with competitor cells (CD45.1^{+}) only had low levels of residual endogenous CD45.2 chimerism (<5%, data not shown). The mean chimerism of mice transplanted with *FancC +/+* cells 1 month after transplantation ranged from 15% when 100 Sca1^{+} lin$^{-/dim}$ cells were transplanted to 55% chimerism when 5,000 Sca1^{+} lin$^{-/dim}$ cells were transplanted. In contrast, the mean chimerism of *FancC −/−* cells over these same Sca1^{+} lin$^{-/dim}$ concentrations ranged from 3% to a maximum of 12%.

Long-term repopulating ability of the two test cell populations was determined by evaluating CD45.2 chimerism in peripheral blood cells of recipient mice 6 months after transplantation. A dose-dependent increase in donor chimerism of mice transplanted with *FancC +/+* and *FancC −/−* cells was observed. A mean chimerism of 59% was detected in recipient mice transplanted with the highest cell dose of *FancC +/+* cells. While there was an increase in chimerism in the recipients of *FancC −/−* donor cells, only a 10% peripheral blood chimerism was achieved in the recipients transplanted with the highest number of Sca1^{+} lin$^{-/dim}$ cells. Together, the data indicate that the short- and long-term repopulating ability is markedly reduced in *FancC −/−* hematopoietic stem cells over a wide range of input test cells.

FancC −/− Sca1^{+} lin$^{-/dim}$ cells have decreased RU activity. To compare the repopulating ability between *FancC −/−* and *FancC +/+* hematopoietic cells in a quantitative fashion, RU activity was calculated as described.[27,28] Table 1 contains the RU calculations for two independent experiments. In both experiments, the *FancC −/−* hematopoietic cells had a lower repopulating ability as compared with *FancC +/+* hematopoietic cells at each monthly analysis. By 4 months after transplantation, there was a 9-fold to 12-fold difference in RU activity of BM cells from the *FancC +/+* and *FancC −/−* mice.

Multilineage analysis of FancC −/− and FancC +/+ hematopoietic cells in reconstituted mice. To determine whether mice transplanted with *FancC −/−* donor cells exhibited any evidence of a preferential repopulation defect in myeloid cells, we evaluated the peripheral blood samples for test cell chimerism using a series of antibodies that are specific to myeloid or lymphoid cells. This was accomplished by dual staining cells for simultaneous expression of CD45.2 (test cell population) and specific lineage markers. The lineages examined by two-color fluorescence cytometry included T cell (CD3), B cell (B220), granulocyte (Gr1), and monocyte/macrophage (Mac1). The summary of multilineage analysis for a representative experiment 5 months posttransplantation is shown in Table 2. Although there were significant differences in

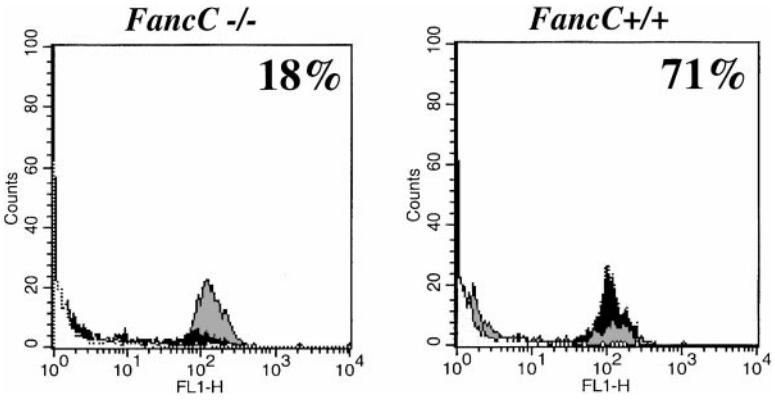

Fig 3. Chimerism of representative irradiated recipient mice transplanted with *FancC −/−* or *FancC +/+* BM cells 6 months after transplantation. Representative histograms of peripheral blood cells obtained from recipient mice transplanted with 500,000 low-density competitor cells expressing the CD45.1 antigen and 5,000 Sca1^{+}lin$^{-/dim}$ *FancC −/−* cells (left panel) or 5,000 Sca1^{+}lin$^{-/dim}$ *FancC +/+* cells (right panel), which express the CD45.2 antigen. Nucleated cells from aliquots of each specimen were stained with antibodies to CD45.1 (▨), CD45.2 (■), and the isotype control (□). The contribution of *FancC −/−* and *FancC +/+* cells to the chimerism is indicated in the upper righthand corner of each histogram.

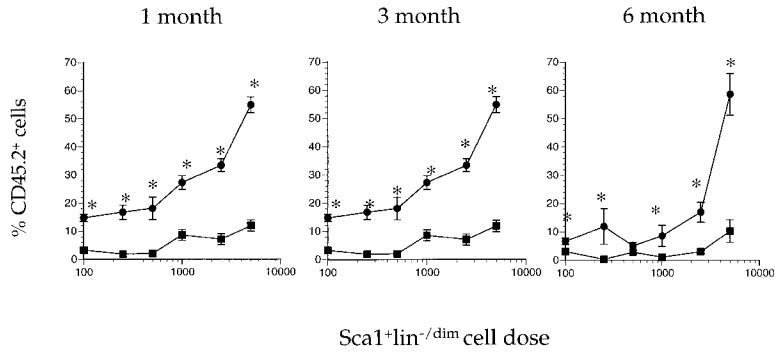

Fig 4. Summary of chimerism in recipient mice transplanted with *FancC* −/− or *FancC* +/+ test cells. Chimerism of peripheral blood cells was determined by fluorescence cytometry for mice transplanted with *FancC* −/− (■) or *FancC* +/+ (●) Sca1$^+$lin$^{-/dim}$ cells and 500,000 competitor cells. The mean chimerism for each Sca1$^+$lin$^{-/dim}$ cell number transplanted was calculated for each genotype, and the data generated at 1, 3, and 6 months after transplantation are summarized in the graphs as indicated. The error bars represent standard error of the means. Twelve mice (six per genotype) were transplanted at each test cell concentration. *$P < .05$ *FancC* −/− cells exhibit a significant decrease in repopulating ability as compared with *FancC* +/+ cells over several Sca1$^+$lin$^{-/dim}$ cell doses transplanted at every time point evaluated.

the repopulating ability of *FancC* −/− and *FancC* +/+ test cells, the data demonstrate that *FancC* −/− cells contribute equally to lymphoid and myeloid lineages. We conclude that these data are consistent with a defect in the pluripotential hematopoietic stem cell compartment.

DISCUSSION

FA is the most common genetic cause of BM failure.[2] Currently, the only cure for the progressive aplasia found in the high majority of FA patients is allogeneic BM transplantation. Many investigators have demonstrated that there is a defect in the hematopoietic progenitor compartment in FA patients.[15-17] However, progenitor assays, which grow in culture over a few weeks, do not correlate with in vivo stem cell function. Therefore, while the observation that FA patients acquire BM failure suggests a defect in the stem cell compartment, a formal experimental proof of this hypothesis in human stem cells has not been conducted. Further, the evaluation of human hematopoietic stem cell function experimentally is difficult due to the lack of a quantitative in vitro assay and inherent problems in transplanting human cells into xenograft systems.

Murine models provide powerful tools for basic research and answering clinical questions that cannot be practically or ethically addressed in human systems. We used a murine model of *FancC* to determine whether *FancC* −/− hematopoietic stem cells have reduced repopulating ability in vivo as compared with *FancC* +/+ cells using a competitive repopulation assay. A key feature of this assay is that the result measures a functional ability of the hematopoietic stem cell and does not rely on phenotypic determinants of output cells. The assay is extremely sensitive to even small differences in test cell populations because evaluation of the entire differentiation pathway is analyzed. In addition, the use of a wide series of limiting dilutions of test cells further strengthens the sensitivity of this assay. Our data in two independent experiments indicate that *FancC* −/− hematopoietic stem cells have a marked early, as well as late, repopulation defect over a 50-fold range of test cells.

To quantitatively assess the relative difference in repopulating ability between *FancC* −/− and *FancC* +/+ cells, we calculated a repopulating unit activity from recipients who received 5 to 10,000 Sca1$^+$ lin$^{-/dim}$ test cells in two independent experiments. These cell doses were chosen because the low chimerism detected in mice transplanted with fewer *FancC* −/− cells would introduce significant error in the calculation. Calculated RU activity values generated from donor *FancC* +/+ cells and transplanted into C57Bl/6 recipients were comparable to previously published data for normal C57Bl/6 marrow cells.[27] A 7-fold to 9-fold reduction in early repopulating ability and a 9-fold to 12-fold reduction in late repopulating

Table 1. Short- and Long-Term Repopulating Ability of Sca1$^+$lin$^{-/dim}$ Donor Cells From *FancC* −/− and *FancC* +/+ Mice

	Time (mo)					
Genotype	1	2	3	4	5	6
Experiment 1						
−/−	0.7 ± 0.1*	0.9 ± 0.2*	1.2 ± 0.3*	0.9 ± 0.2*	0.7 ± 0.2*	0.8 ± 0.3*
+/+	6.3 ± 0.7	8.0 ± 1.0	8.1 ± 1.4	8.1 ± 1.9	8.6 ± 2.2	8.3 ± 2.3
Fold Δ	9	9	7	9	12	10
Experiment 2						
−/−	0.4 ± 0.1*	0.3 ± 0.1*	0.1 ± 0.1*	0.1 ± 0.1*	0.1 ± 0.1*	0.2 ± 0.1*
+/+	2.6 ± 0.2	1.1 ± 0.3	1.0 ± 0.2	1.2 ± 0.2	1.0 ± 0.2	1.7 ± 0.4
Fold Δ	7	4	10	12	10	9

Repopulating units are expressed as mean ± SEM for six mice per group.

Abbreviation: SEM, standard error of the mean.

*$P \leq .05$.

Table 2. Donor Contribution to Multiple Lineage Repopulation 5 Months After Transplantation

Genotype	B220	CD3	GR1	Mac1	Total
Experiment 1					
−/−	8 ± 3*	6 ± 2*	18 ± 5*	12 ± 5*	13 ± 3*
+/+	60 ± 10	46 ± 9	57 ± 7	62 ± 7	63 ± 7

Results are expressed as mean ± SEM for six mice per group. The values displayed represent the proportion of cells in individual lineages that are donor cell derived.

*$P \leq .05$.

ability was detected in recipient mice transplanted with *FancC* −/− cells.

The test cell chimerism detected in experiment 2 was lower than the chimerism noted in experiment 1 (Table 1). Potential explanations for the lower levels of chimerism detected in experiment 2 may relate to variability between the two experiments resulting from differences in test or competitor cell populations (ie, ficoll-hypaque separation, $Sca1^+$ $lin^{-/dim}$ cell purification) or differences in the response of the hosts to the conditioning regimen. Two experimental controls were used to evaluate this latter possibility. First, we showed that a lethal irradiation dosage was administered to both CD45.1 and CD45.2 recipients. All irradiated mice that did not receive exogenous cells died 10 to 14 days after irradiation. Second, we included a set of control animals (CD45.2) that were transplanted with only competitor cells (CD45.1) to examine whether residual endogenous hematopoiesis contributed significantly to the measured chimerism. Another plausible explanation for the differences between experiments 1 and 2 is that, although CD45.1 and CD45.2 are isoenzymes, these antigens could potentially induce a weak immune response when transplanted into hosts with disparate CD45 antigen expression. Other investigators have also suggested this possibility using milder conditioning regimens of the recipient animals.[33] However, despite the difference in absolute test cell chimerism between the two experiments, the relative difference in repopulating ability between *FancC* −/− and *FancC* +/+ test cells was similar in both experiments.

The hematopoietic system is characterized by a hierarchy of multiple compartments (ie, stem, progenitor, and differentiated cell compartments), as well as cell lineages (ie, myeloid, lymphoid, and erythroid). Loss of specific gene products may affect some lineages and compartments, but not others. In FA patients, for instance, there is a selective attrition of myeloid cells in vivo, while lymphocyte populations remain normal, and patients with FA are highly predisposed to myeloid, but not lymphoid leukemias. We used the competitive repopulation assay to determine whether *FancC* is required for normal lymphoid, as well as myeloid cell repopulation. While we were able to detect profound differences between the repopulating ability of *FancC* −/− and *FancC* +/+ test cells, no differential requirement for *FancC* was detected between myeloid or lymphoid lineages. Our observation that recipient mice transplanted with *FancC* −/− cells have an equivalent repopulation defect in multiple lineages is consistent with this gene being important in hematopoietic stem cell function in *FancC* −/− mice.

Murine models of *FancC* have recently been used to begin to delineate the consequences of loss of *FancC* function in differentiated hematopoietic cells, as well as in hematopoietic progenitor cells. Differentiated splenic lymphocytes from *FancC* −/− mice were shown to exhibit increased spontaneous, as well as induced chromosomal aberrations, similar to lymphocytes from FA patients.[18] Further, *FancC* −/− hematopoietic progenitors are hypersensitive to MMC[20,21] and inhibitory cytokines,[19,20] including interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and macrophage inflammatory protein-1α. In addition, loss of *FancC* predisposes progenitors to inhibitory cytokine-mediated apoptosis.[20,22] Other studies evaluating the hematologic consequences of in vivo administration of MMC demonstrated that *FancC* −/− mice develop pancytopenia, BM aplasia, and death after serial weekly intraperitoneal injections of MMC for 3 to 8 weeks.[21] These studies show that hematopoietic cells from *FancC* −/− mice are hypersensitive in vivo, as well as in vitro, to bifunctional alkylating agents, analogous to human FA blood and BM cells. Together, the previous studies and our present results demonstrate that loss of *FancC* results in a profound alteration in hematopoietic cell function in multiple hematopoietic compartments.

Recent discoveries have now resulted in the positional identification of four FA complementation types and the cloning of three of the cDNAs.[8-10,12] These genes account for the genetic abnormalities detected in approximately 65% to 70% of known FA patients. The isolation of the cDNAs, continued improvements in somatic gene transfer technology, and an improved understanding of the role of these proteins in normal cellular homeostasis[22,34-38] may allow new pharmacologic and gene transfer approaches to ameliorate or cure the hematopoietic disease. Our studies demonstrating a clear repopulating defect in *FancC* −/− hematopoietic stem cells will allow use of the competitive repopulation assay to determine whether *FancC* gene transfer will restore normal proliferation to *FancC* −/− cells in vivo. Such studies will have direct application regarding the ability to correct the proliferation of human hematopoietic stem cells in FA patients.

If gene transfer corrects the biochemical defect and restores normal proliferation to *FancC* −/− hematopoietic stem cells, two questions regarding the in vivo consequences of transduced stem cells will need to be addressed. The first question is: what are the conditions in which the genetically corrected cells will engraft and proliferate? The potential of transduced FA hematopoietic stem cells to engraft and proliferate in the absence of myeloablation is particularly important, as FA patients are hypersensitive to many chemotherapeutic agents and may be at an increased risk for secondary malignancies. It has been previously determined in the murine system that wild-type cells will engraft in the absence of myeloablation when high numbers of donor cells are used.[39,40] In addition, other investigators have determined that significantly higher chimerism of donor cells is obtained in nonmyeloablated normal recipients when mobilized peripheral blood or BM cells are used.[26,41] It will be important to evaluate the engraftment and in vivo proliferation potential of transduced *FancC* −/−, BM, and mobilized peripheral blood stem cells after transplantation into nonmyeloablated *FancC* −/− mice.

Second, gene transfer, using current strategies, will result in transduction of only a small subpopulation of the total number

of hematopoietic stem cells. It remains to be determined what the fates of the untransduced stem cells, as well as the endogenous stem cells, will be after transplantation. The noncorrected cells may remain quiescent, while the genetically corrected cells maintain hematopoiesis. There is now precedence for this pattern of clonal proliferation occurring in an FA patient where a somatic reversion resulted in a single stem/progenitor cell restoring normal hematopoietic cell function for at least 5 years.[42] Alternatively, the population of untransduced stem cells may undergo further damage leading to apoptosis or leukemic transformation. The *FancC* −/− mice will provide a valuable model system to evaluate these basic questions.

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