Original Research

Age-related decline of mast cell regeneration in senescence-accelerated mice (SAMP1) after chemical myeloablation due to senescent stromal cell impairment

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Abstract

An age-related decline in immune functions is referred to as immunosenescence. Mast cells play an important role in the immune system. However, it has not yet been determined if aging may affect mast-cell development. In the present study, we examined the age-related change in mast-cell development after myeloablation with 5-fluorouracil (5-FU) in senescence accelerated mice (SAMP1), which exhibit senescence-mimicking stromal cell impairment after 30 weeks of age. We found that aged mice with stromal cell impairment (30–36 weeks old) showed a lower recovery of the number of femoral mast-cell progenitors (colony-forming unit [CFU]-mast) (64% of steady state), whereas young mice (8–12 weeks old) showed a higher recovery (122% of steady state). Stromal cells influence mast-cell development by producing positive regulators such as stem cell factor (SCF) and negative regulators such as transforming growth factor-beta (TGF-β). The ratio of the gene expression of SCF to that of TGF-β (SCF/TGF-β ratio) indicates the balance of positive and negative regulation of mast-cell development. SCF/TGF-β ratio increased in both the young and aged mice after 5-FU treatment. However, the SCF/TGF-β ratio rapidly decreased in aged mice, whereas it remained high in young mice. The number of femoral CFU-mast in the S-phase after 5-FU treatment reflects the activation of positive-dominant regulation for mast-cell development by stromal cells. Aged mice showed lower recovery of the number of femoral CFU-mast in the S-phase (47% of steady state), whereas young mice showed a higher recovery (205% of steady state). These results suggest that mast-cell development declines with aging due to stromal-cell functional impairment, which contributes to immunosenescence.

Keywords: aging, mast cell, stromal cell, cytokine, cell cycle

Experimental Biology and Medicine 2012; **237**: 1289–1297. DOI: 10.1258/ebm.2012.012158

Introduction

Aging of the immune system, termed 'immunosenescence', is characterized by a time-dependent functional decline of immunity.[1,2] The complex process of immunosenescence affects both the innate and the adaptive arms of the immune system, resulting in susceptibility to viral and bacterial infections. Mast cells are crucial effecter cells that play a key role in innate immune responses.[3] Although studies are limited, mast cell numbers and functional properties have also been shown to exhibit age-related changes.[4–7] However, it has not yet been fully determined if mast-cell production is affected by aging.

Stromal cells are a heterogeneous collection of mesenchymal stem cells, which are an essential component of the hematopoietic microenvironment. Stromal cells regulate the proliferation and differentiation of hematopoietic stem cells by producing diffusible factors and through direct cellular interactions with adherent molecules.[8,9] Mast cells, like other immune component cells such as T-cells, B-cells and macrophages, are derived from hematopoietic stem cells.[10,11] Mast-cell development in bone marrow (BM) is regulated by stromal cells, which produce positive regulators such as stem cell factor (SCF) and negative regulators such as transforming growth factor-beta (TGF-β).[12]

B lymphopoiesis is predominantly decreased in aged mice as compared with young mice, whereas myelopoiesis is maintained in aged mice at the same level as that in young mice.[12,13] Decreased B-cell production with aging is not only due to functional defects in stem cells but also

due to defects in stromal cell function.[14–16] Thus, it is interesting to investigate whether mast-cell production in the BM may also be decreased with aging due to the deteriorated function of senescent stromal cells in the same manner as senescent B lymphopoiesis.

We recently demonstrated that senescence-accelerated mice (SAMP1) exhibit premature senescence-like stromal cell impairments after 30 weeks of age.[12,16,17] In the present study, we used this mouse model of stromal cell impairment to clarify the effect of senescence-associated defects in hematopoietic microenvironment functions on mast-cell development. Furthermore, we examined mast-cell development under aggravated perturbed conditions, that is, under more sensitive condition induced by 5-fluorouracil (5-FU)[18,19] because steady-state mast-cell development sometimes cannot be observed and seems to be maintained with a sufficient reservoir of cytokines. In other words, mast-cell development may be regulated by low-level cytokine secretion with minimum changes.

Here, we compared the recovery of mast-cell progenitors (CFU-mast) and also simultaneously monitored the time course of gene expression levels for SCF and TGF-β by stromal cells of hematopoietic tissues in young and aged mice treated with 5-FU. Furthermore, we also compared the recovery process of mast-cell progenitor cells in the S-phase in the hematopoietic tissues between young and aged mice to reveal the balance of positive and negative regulation for mast-cell development.

Materials and methods

Mice

Senescent stromal-cell-impaired SAMP1 mice (Japan SLC, Co. Ltd, Hamamatsu, Japan) were used.[20] All mice were housed under specific pathogen-free conditions at $24 \pm 1^{\circ}C$ and $55 \pm 10\%$ relative humidity, under a 12-h light–dark cycle. Autoclaved tap water and food pellets were provided *ad libitum*. The number of splenic cells and splenic hematopoietic progenitor cells in SAMP1 mice starts to decrease significantly at approximately 30 weeks of age, and SAMP1 mice exhibit stromal cell impairment after 30–36 weeks of age.[5,21] In this study, we compared 8- to 12-week-old male SAMP1 mice (designated as young mice) with 30- to 36-week-old male SAMP1 mice (designated as aged mice). All protocols involving laboratory mice were reviewed by a peer-review panel, the Interdisciplinary Monitoring Committee for the Right Use and Welfare of Experimental Animals, at the Nihon University School of Medicine (NUSM) of Japan and approved by the Committee for Animal Care and Use at NUSM (experimental code AP09M003-2). All experiments were performed humanely in strict accordance with NUSM's Guidelines for the Care and Use of Laboratory Animals.

5-FU administration

5-FU (Kyowakirin, Tokyo, Japan), 50 mg/mL, was diluted with pyrogen-free saline to a final concentration of 25 mg/mL. Mice were injected intravenously with 5-FU in a single dose (150 mg/kg body weight).[14,22,23] Mice were evaluated on days 1–7, 10, 14, 21 and 28 after 5-FU treatment. A control group of mice was injected with the same volume of pyrogen-free saline (i.e. 6 mL/kg body weight). Three mice per group were examined at each time point.

Preparation of BM cells and splenic cells

BM cell suspensions were prepared by repeatedly flushing the cells from femurs. Cells were dispersed by repeated passage through a 23-gauge hypodermic needle with α-minimum essential medium (α-MEM; Life Technologies, Grand Island, NY, USA) or Iscove's modified Dulbecco's medium (IMDM; Invitrogen Corp., Carlsbad, CA, USA). BM cells from the bilateral femora of three mice per experimental group were individually removed, and their blood parameters were separately determined. Splenic tissue samples were minced in ice-cold α-MEM or IMDM and gently homogenized with a Potter's glass homogenizer to obtain a single-cell suspension. For colonization assays, BM cells and splenic cells were pooled and assayed (see below).

In vitro colony formation assays

The colony formation assay with colony-forming unit-mast (CFU-mast) cells has been previously described.[12,24] Briefly, BM cells or splenic cells were cultured in 35-mm plastic dishes (Falcon, Becton Dickinson, Franklin Lakes, NJ, USA) in 2 mL of semisolid α-MEM containing 0.33% Bacto-Agar (Difco Laboratories, Detroit MI, USA), 25% horse serum (JRH Bioscience, Lenexa, KS, USA) and 20% STIL (interleukin-3-producing T-cell line)-conditioned medium as a source of interleukin-3. Colony formation by colony-forming unit-granulocyte-macrophage (CFU-GM) cells was assayed using a semisolid medium containing granulocyte macrophage colony stimulating factor (GM-CSF; R&D, Minneapolis, MN, USA). BM cells or splenic cells were cultured in 35-mm plastic Petri dishes containing 1 mL of MethoCult M 3231 medium (Stem Cell Technologies Inc., Vancouver, BC, Canada), which consisted of IMDM composed of 1% methylcellulose, supplemented with 30% fetal bovine serum (FBS), 1% bovine serum albumin, 0.1 mmol/L 2-mercaptoethanol, 2 mmol/L L-glutamine and 10 ng/mL GM-CSF.[19] Triplicate culture plates containing equal amounts of CFU-mast and CFU-GM cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Aggregates of 50 or more cells were counted as colonies on day 21 for CFU-mast and day 7 for CFU-GM. Fibroblast colony colony-forming unit (CFU-F) was assayed in α-MEM containing 20% FBS (JRH Bioscience).[5] Aggregates of 50 or more cells were counted as colonies on day 10 for CFU-F after staining with May–Grunwald–Giemsa stain.[5]

Hydroxyurea suicide experiments to count CFU-mast cells in the S-phase

Equal volumes of cell suspension were placed into two test tubes. Hydroxyurea (HU) (Sigma-Aldrich, St Louis,

MO, USA) was dissolved in α-MEM and added to one tube at a final concentration of 6×10^{-3} mol/L.[25,26] α-MEM alone was added to the control. Both tubes were incubated for one hour at 37°C. After washing three times with α-MEM, the CFU-mast assay was performed. The proportions of cells in the S-phase among CFU-mast cells were calculated using the following formula: non-treated colony number − HU-treated colony number/non-treated colony number. The results are expressed as percentages.

Total RNA extraction and quantitative realtime polymerase chain reaction analysis

Total RNA was extracted from pooled BM cells or splenic cells from three mice per group using ISOGEN reagent (Nippongene Corp., Toyama, Japan) in accordance with the manufacturer's instructions. The isolated mRNA was reverse-transcribed using Superscript III (Life Technologies) and Oligo-dT (Promega Corp., Madison, WI, USA). Next, triplicate of the transcribed cDNA per sample were analyzed by quantitative realtime polymerase chain reaction (PCR). Reactions were performed with TaqMan™ Universal Fast PCR master mix (Applied Biosystems, Foster City, CA, USA) and specific primers and probes for murine SCF, TGF-β and glyceraldehyde phosphate dehydrogenase (GAPDH) (Applied Biosystems; TaqMan™ Gene Expression Assays; SCF, Mm00442972_m1; TGF-β, Mm00441724_ml; GAPDH, Mm99999915_g1) using the Applied Biosystems 7900 Sequence Detection System. PCR conditions and data analysis were in accordance with the instructions provided with the Sequence Detection System, version 2.0. All reactions were performed in triplicate. In accordance with the manufacturer's instructions, cytokine-specific signals were normalized by GAPDH signals using the formula $2^{-\Delta Ct} = 2^{-(Ct\ GAPDH - Ct\ Cytokine)}$, and the relative level of cytokine gene expression was calculated using $2^{-\Delta\Delta Ct} = 2^{-(\Delta Ct,\ for\ 5\text{-}FU\ treatment - \Delta Ct,\ for\ control)}$.

Statistical analysis

All data are presented as mean ± standard deviation (SD) or mean ± standard error (SE). The significance of differences among experimental groups was assessed using one-way analysis variance (ANOVA). The cut-off value used to determine statistical significance was $P < 0.05$.

Results

Changes in the number of CFU-GM and CFU-mast cells in the BM and spleen of young and aged mice after 5-FU treatment

Figure 1a shows the changes in numbers of CFU-GM cells in the BM of young and aged mice. The absolute numbers of CFU-GM in the femoral BM of untreated young and aged mice are 36,729 ± 2152 and 43,915 ± 1117 (mean ± SD), respectively. The number of CFU-GM in young mice treated with 5-FU decreases rapidly to 1.6% of pretreatment levels by day 2 and promptly recovers and overshoots to 156% of pretreatment levels by day 10, followed by a decrease to 82% of pretreatment levels by day 28. The changes in CFU-GM cell numbers in treated aged mice parallel the changes in young mice.

Figure 1b shows the changes in the numbers of CFU-mast cells in the BM of young and aged mice. The absolute numbers of CFU-mast cells in the femoral BM of untreated young and aged mice are 18,550 ± 1071 and 14,527 ± 726 (mean ± SD), respectively. Concerning the recovery of progenitor cells, changes in the number of CFU-mast cells in young and aged mice are significantly different from changes in the number of CFU-GM in young and aged mice (Figure 1a versus b). Young and aged mice show only a limited difference in the CFU-GM number (Figure 1a). Regarding the CFU-mast cell number, young mice show a higher recovery (122% of steady state) than aged mice (64% of steady state) (Figure 1b). The recoveries of CFU-mast cell number in the BM of young and aged mice are delayed compared with the recovery of CFU-GM cell number in young and aged mice.

Figure 2a shows the changes in the numbers of CFU-GM cells in the spleens of young and aged mice. The absolute number of CFU-GM in the spleens of untreated young and aged mice is 24,587 ± 2360 and 6754 ± 640 (mean ± SD), respectively. The number of CFU-GM in treated young mice decreases rapidly to 0.04% of pretreatment levels by day 1 and then promptly recovers and overshoots to 668% of pretreatment levels by day 14, followed by a decrease to pretreatment levels by day 28. The number of CFU-GM in treated aged mice decreases rapidly to 0.24% of pretreatment levels by day 1 and then promptly recovers and overshoots to 506% of pretreatment levels by day 10, followed by a decrease to pretreatment levels by day 28.

Figure 2b shows the changes in the numbers of splenic CFU-mast cells in young and aged mice. The absolute number of splenic CFU-mast cells in untreated young and aged mice is 21,345 ± 2988 and 4892 ± 742 (mean ± SD), respectively. Concerning the recovery of progenitor cells, changes in the number of CFU-mast cells are different between young and aged mice. Regarding the CFU-mast cell number, young mice show a higher recovery (200% of steady state) than aged mice (61% of steady state) (Figure 2b). The recovery of CFU-mast cell number in the spleens of young and aged mice is delayed compared with the recovery of CFU-GM cell number in young and aged mice.

Changes in gene expression levels of SCF and TGF-β and the ratio of gene expression of SCF to that of TGF-β in the BM of young and aged mice after 5-FU treatment

To reveal the effect of stromal cells on the regeneration of mast-cell progenitors in young and aged mice after 5-FU treatment, we evaluated the gene expression levels of SCF, a positive regulator of mast-cell development, and TGF-β, a negative regulator. Notably, both SCF and TGF-β are produced by stromal cells in the BM and spleen.[12,27,28]

Figures 3a and b show the changes in gene expression levels of SCF and TGF-β in the BM of young and aged

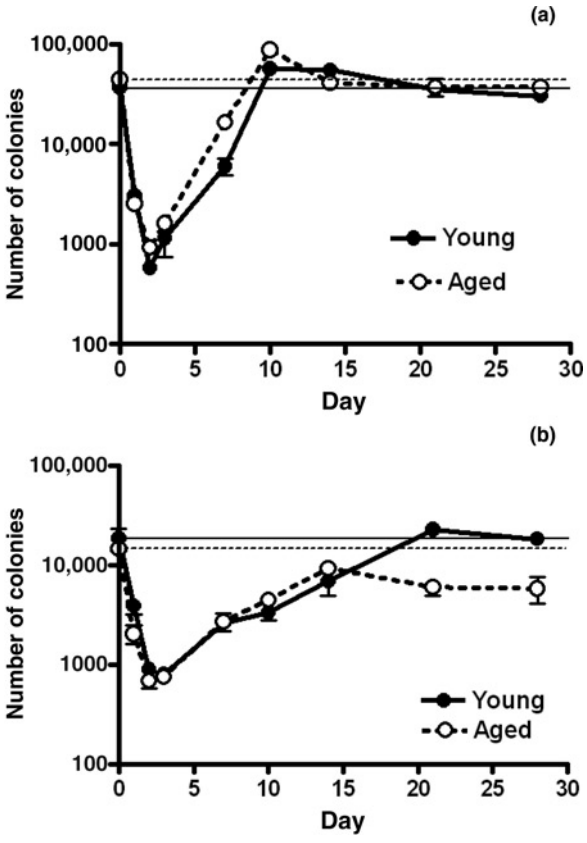

Figure 1 Time course of changes in the cell number of hematopoietic progenitors in the bone marrow of young and aged mice treated with 5-fluorouracil (5-FU). Time course of changes in the number of colony-forming unit (CFU)-granulocyte-macrophage (a) and CFU-mast (b) in bone marrow of young mice and aged mice following 5-FU treatment. Samples were obtained from three mice 1, 2, 3, 7, 14, 21 or 28 days after a single intravenous injection of 150 mg/kg body weight of 5-FU. Each bar represents the mean ± SD obtained from triplicate experiments. The horizontal solid line and dotted line indicate the mean number of hematopoietic progenitor cells of untreated young mice and untreated aged mice, respectively

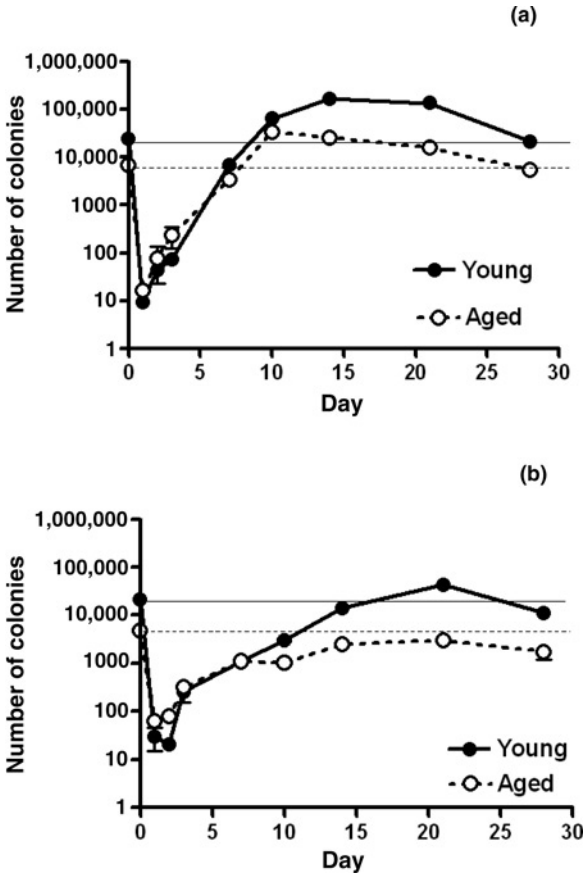

Figure 2 Time course of changes in the cell number of hematopoietic progenitors in the spleen of young and aged mice treated with 5-fluorouracil (5-FU). Time course of changes in the number of colony-forming unit (CFU)-granulocyte-macrophage (a) and CFU-mast (b) in the spleens of young and aged mice following 5-FU treatment. Samples were obtained from three mice 1, 2, 3, 7, 14, 21 or 28 days after a single intravenous injection of 150 mg/kg body weight of 5-FU. Each bar represents the mean ± SD obtained from triplicate experiments. The horizontal solid line and dotted line indicate the mean number of hematopoietic progenitor cells of untreated young mice and untreated aged mice, respectively

mice. The levels of SCF in the BM of untreated young and aged mice are much lower than those of TGF-β (0.036 and 0.016, respectively, relative to the expression level of TGF-β in untreated mice, which is set at 1.0) (Figures 3a and b). The relative gene expression levels of SCF against those of TGF-β are two-times lower in aged mice as compared with young mice.

Gene expression levels of SCF and TGF-β in the BM of young mice after 5-FU treatment are shown in Figure 3a. Prior to treatment with 5-FU, SCF gene expression levels are 28 times lower than TGF-β expression levels. After treatment, the expression of SCF in the BM increases continuously and reaches 5718% of pretreatment levels by day 4. This peak level is maintained for a couple of days. Then, the level decreases continuously, reaching 195% of pretreatment levels on day 21, followed by an increase to 636% of pretreatment levels on day 28. The gene expression levels for TGF-β in the BM increase continuously to 766% of pretreatment levels by day 4 and then decrease continuously to 88% of pretreatment levels by day 10, followed by a slight increase to 142% of pretreatment levels.

Gene expression levels of SCF and TGF-β in the BM of aged mice after 5-FU treatment are shown in Figure 3b. Initially, SCF gene expression levels are 63 times lower than the levels of TGF-β gene expression. After 5-FU treatment, the expression of SCF in the BM continuously increases and reaches 10,312% of pretreatment levels by day 5. Then, the level rapidly decreases to 247% of pretreatment levels by day 7, after which it slightly increases again to 384% of pretreatment levels followed by a decrease to 161% of pretreatment levels. The changes in TGF-β mRNA levels are essentially parallel in aged mice and young mice.

To elucidate the difference between young and aged mice in terms of the balance of positive and negative regulation of mast-cell development, it was necessary to evaluate our model from a different perspective. Therefore, we determined the changes in the ratio of the gene expression of SCF to that of TGF-β (SCF/TGF-β ratio) after treatment with 5-FU (Figures 3c and d).

In the BM of young mice (Figure 3c), the SCF/TGF-β ratio is 0.036 prior to treatment. After treatment with 5-FU, the SCF/TGF-β ratio increases by 694% of pretreatment levels by day 2 and remains high from days 2 through 10; then, the ratio decreases slowly to 152% of pretreatment levels

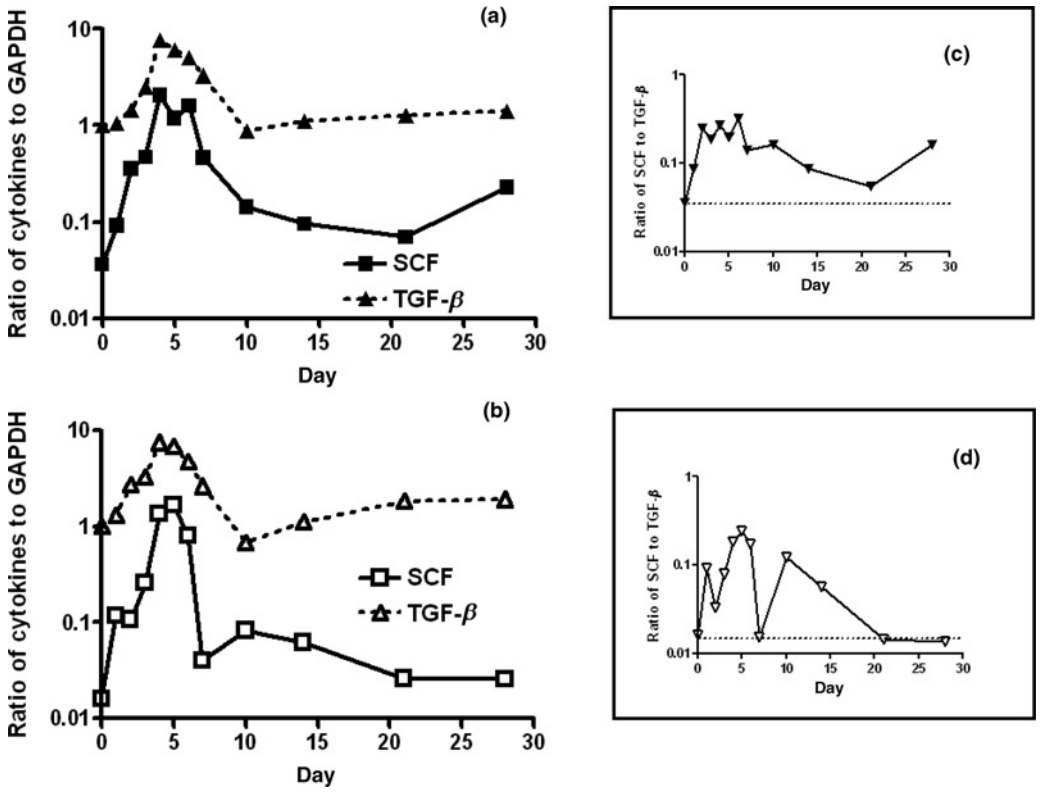

Figure 3 Time course of changes in the gene expression levels of stem cell factor (SCF) and transforming growth factor-beta (TGF-β) and the relative gene expression levels of SCF in the bone marrow of young and aged mice following treated with 5-fluorouracil (5-FU). SCF and TGF-β mRNA levels in bone marrow of young (a) and aged mice (b) were evaluated on days 1–7, 14, 21 or 28 after a single intravenous injection of 150 mg/kg body weight of 5-FU. The results were normalized to glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA levels. The values shown for SCF are relative to the TGF-β level in untreated young or aged mice, which was arbitrarily set at a value of 1. The relative gene expression level of SCF, which is the SCF gene expression level divided by the TGF-β gene expression level, in the bone marrow of young (c) and aged mice (d) was evaluated. The horizontal dotted line indicates the relative gene expression levels of SCF in untreated young (c) and aged (d) mice

by day 21, followed by an increase to 444% of pretreatment levels by day 28. In the BM of aged mice (Figure 3d), the SCF/TGF-β ratio in pretreated mice is 0.016. After treatment with 5-FU, the ratio slowly increases to 1500% of pretreatment levels on day 5, after which the ratio shows deep oscillatory changes followed by a rapid decrease to the pretreatment levels.

Changes in gene expression levels of SCF and TGF-β and the ratio of gene expression of SCF to that of TGF-β in the spleens of young and aged mice after 5-FU treatment

Figure 4 shows the changes in gene expression levels of SCF and TGF-β in the spleens of young and aged mice. The gene expression levels of SCF in the spleens of untreated young and aged mice are much lower than the gene expression levels of TGF-β (0.020 and 0.084, respectively, relative to the expression level of TGF-β in untreated mice, which is set at 1.0) (Figures 4a and b). The SCF/TGF-β ratio in aged mice is four times higher than the ratio in young mice.

Gene expression levels of SCF and TGF-β in the spleens of young mice after 5-FU treatment are shown in Figure 4a. Prior to treatment, the SCF gene expression levels are 50 times lower than those of TGF-β. The gene expression levels for SCF in the spleen temporally decrease to 67% of pretreatment levels during the first 24 h after 5-FU treatment. Then, the levels continuously increase to 293% of pretreatment levels by day 6, followed by a decrease to 123% of pretreatment levels by day 14 and then an increase to 215% of pretreatment levels by day 28. The change in the TGF-β mRNA levels in the spleens of young mice is almost parallel to the changes in SCF mRNA levels in the spleen.

Gene expression levels of SCF and TGF-β in the spleens of aged mice after 5-FU treatment are shown in Figure 4b. Prior to treatment, the SCF gene expression level is 12 times lower than that of TGF-β. The gene expression level for SCF in the spleens of aged mice is 3% of pretreatment levels on day 3 and 50% of pretreatment levels on day 5, followed by an increase to 167% of pretreatment levels on day 7, after which the level oscillates between 83% and 278% of the pretreatment level. The change in the TGF-β mRNA level of the spleens of aged mice is almost parallel to the changes in SCF mRNA levels.

In the spleens of young mice (Figure 4c), the SCF/TGF-β ratio is 0.02 prior to treatment with 5-FU. The ratio oscillates between 70% and 105% of pretreatment levels during the first five days after treatment. The ratio increases to 270% of pretreatment levels by day 10, followed by a decrease to 190% of pretreatment levels by day 14, after which the ratio remains unchanged.

In the spleens of aged mice (Figure 4d), the SCF/TGF-β ratio is 0.084 prior to treatment with 5-FU. The splenic SCF/TGF-β ratio in aged mice treated with 5-FU sharply

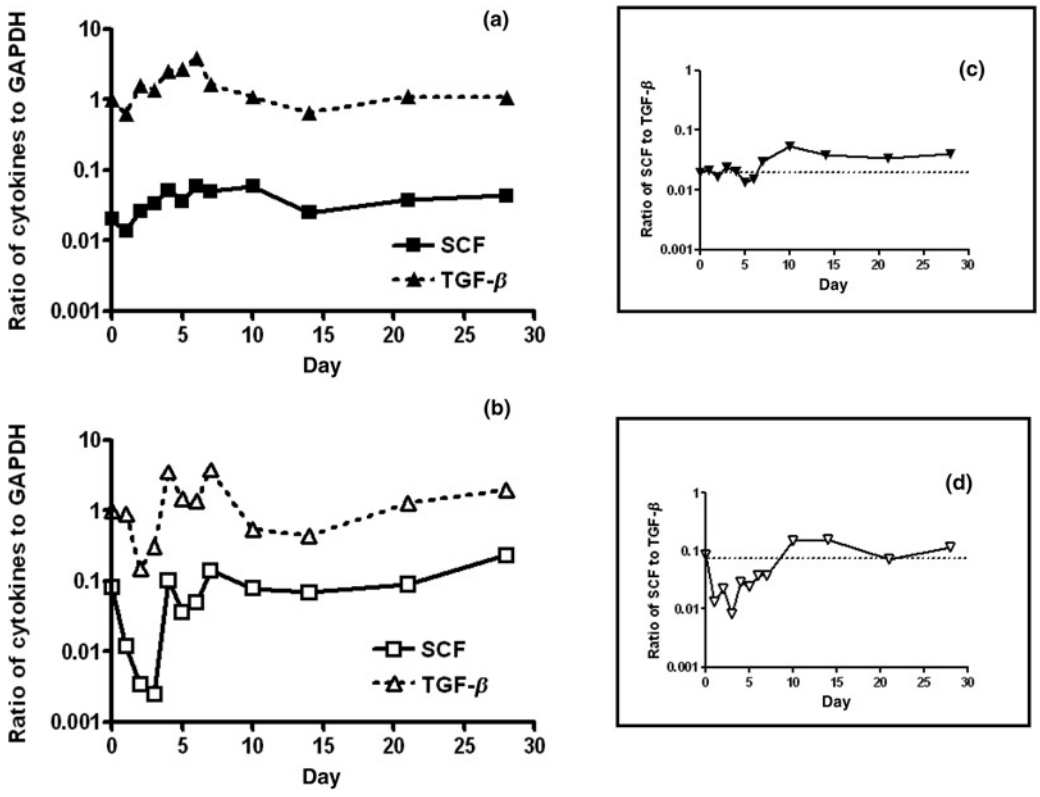

Figure 4 Time course of changes in the gene expression levels of stem cell factor (SCF) and transforming growth factor-beta (TGF-β) in the spleen of young and aged mice treated with 5-fluorouracil (5-FU). SCF and TGF-β mRNA levels in the spleens of young (a) and aged mice (b) were evaluated on days 1–7, 14, 21 or 28 after a single intravenous injection of 150 mg/kg body weight of 5-FU. The results were normalized to glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA levels. The values shown for SCF are relative to the TGF-β level in untreated young or aged mice, which was arbitrarily set at a value of 1. The relative gene expression level of SCF, which is the SCF gene expression level divided by the TGF-β gene expression level, in the spleens of young (c) and aged mice (d) was evaluated. The horizontal dotted line indicates the relative gene expression levels of SCF in untreated young (c) and aged (d) mice

decreases to 15% of pretreatment levels during the first 24 h and oscillates between 10% and 44% of pretreatment levels from days 2 through 7. Then, the ratio increases to 187% of pretreatment levels on day 14 and returns to pretreatment levels on day 21 followed by an increase to 202% of pretreatment levels.

Changes in the number of CFU-mast cells in the S-phase in the BM and spleens of 5-FU-treated mice

Figure 5a shows the changes in numbers of CFU-mast cells in the S-phase in the BM of young and aged mice. The absolute number of CFU-mast cells in the S-phase in the BM of untreated young and aged mice is 1406 ± 159 and 1947 ± 181 (mean $\pm$ SD), respectively. After treatment with 5-FU, the number of CFU-mast cells in the S-phase in the BM of young mice decreases rapidly to 3% of pretreatment levels by day 3 and then recovers and overshoots to 205% of pretreatment levels by day 14; then, it decreases to 159% of pretreatment levels on day 21, followed by an increase to 171% of pretreatment levels by day 28. The number of CFU-mast cells in the S-phase in the BM of treated aged mice decreases rapidly to 5% of pretreatment levels by day 3 and recovers to 47% of pretreatment levels by day 7, followed by a decrease to 33% of pretreatment levels on day 28.

Figure 5b shows the changes in the numbers of CFU-mast cells in the S-phase in the spleens of young and aged mice. The absolute numbers of CFU-mast cells in the S-phase in the spleens of untreated young and aged mice are 3483 ± 394 and 1551 ± 100 (mean $\pm$ SD), respectively. The number of CFU-mast cells in the S-phase in the spleen in treated young mice decreases rapidly to 1% of pretreatment levels by day 3 and recovers and overshoots to 143% of pretreatment levels by day 21, followed by a decrease to 45% of pretreatment levels on day 28. The number of CFU-mast cells in the S-phase in the spleen in treated aged mice decreases rapidly to 1% of pretreatment levels by day 3 and recovers to 35% of pretreatment levels on day 14 and remains unchanged thereafter.

Changes in the number of CFU-F in the BM of young and aged mice after 5-FU treatment

Figure 6 shows the changes in the numbers of CFU-F in the BM of young and aged mice. The absolute numbers of CFU-F in the femoral BM of untreated young and aged mice are 183 ± 14 and 364 ± 5 (mean $\pm$ SD), respectively. The number of CFU-F in treated young mice decreases rapidly to 10% of pretreatment levels by day 3 and then promptly recovers and overshoots to 136% of pretreatment levels by day 7, after which the level oscillates between 92% and 142% of the pretreatment level. The number of CFU-F in treated aged mice decreases rapidly to 35% of pretreatment levels by day 3 and recovers and overshoots to

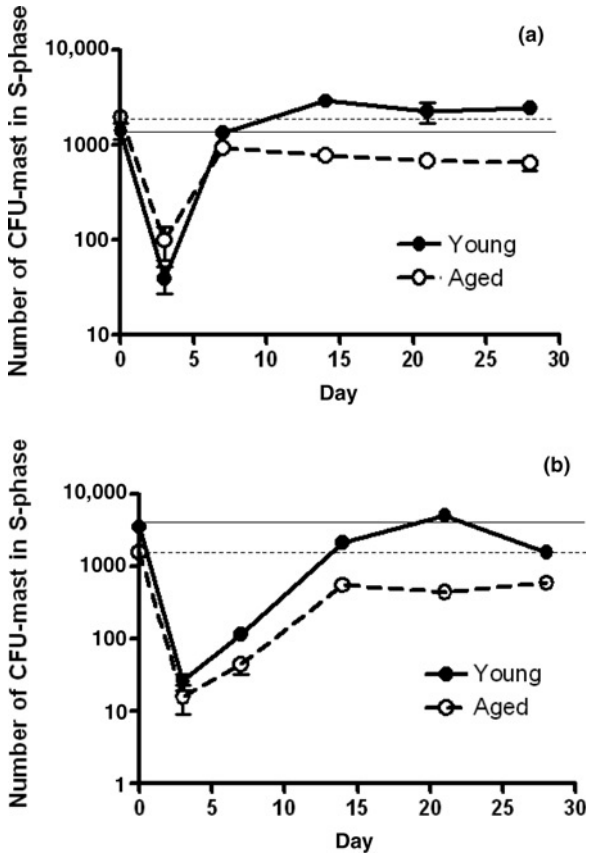

Figure 5 Time course of changes in the number of colony-forming unit (CFU)-mast in the S-phase in the bone marrow and the spleen of mice treated with 5-fluorouracil (5-FU). The number of CFU-mast in the S-phase in the bone marrow (a) and spleen (b) of young and aged mice was evaluated on day 0 (control) and on days 7, 14, 21 and 28 after a single intravenous injection of 150 mg/kg body weight of 5-FU. Each bar represents the mean ± SE obtained from triplicate experiments

135% of pretreatment levels by day 14, after which the level oscillates between 72% and 82% of the pretreatment level.

Discussion

Mast cells are derived from hematopoietic stem cells and are specified during hematopoiesis earlier than and independently from granulocytes; however, unlike other hematopoietic-lineage cells, they do not ordinarily circulate in the peripheral blood in their mature form. Precursor mast cells migrate to the vascularized tissues or serosal cavities where they will ultimately reside and undergo differentiation and maturation in the mucosa.[10,11,29–31]

Studies of a mast-cell-deficient mouse model with the genotype W/W^v and Sl/Sl^d harboring mutations in c-kit and its ligand SCF have provided molecular evidence that the c-kit/SCF signaling pathway is essential for mast-cell development.[32–34] In contrast, the TGF-β signaling pathway negatively regulates mast-cell development.[28,35] Furthermore, TGF-β down-regulates not only SCF production by stromal cells but also c-kit expression on the cell surface of BM hematopoietic progenitor cells.[36,37] Mast-cell development in the BM is regulated by positive regulators such as SCF and negative regulators such as

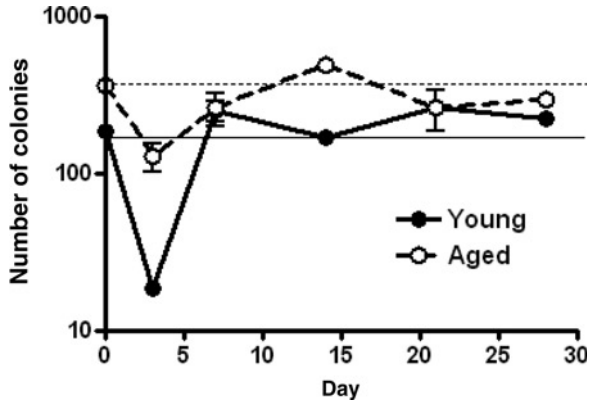

Figure 6 Time course of changes in the cell number of fibroblast colony-forming unit (CFU-F) in the bone marrow of young and aged mice treated with 5-fluorouracil (5-FU). Time course of changes in the number of CFU-F in bone marrow of young mice and aged mice following 5-FU treatment. Samples were obtained from three mice 3, 7, 14, 21 or 28 days after a single intravenous injection of 150 mg/kg body weight of 5-FU. Each bar represents the mean ± SD obtained from triplicate experiments. The horizontal solid line and dotted line indicate the mean number of CFU-F of untreated young mice and untreated aged mice, respectively

TGF-β produced by stromal cells.[19,27] However, it has not been determined if mast-cell development changes with aging due to senescent stromal cell impairment. Thus, we investigated whether age-related changes occur in mast-cell development using SAMP1 mice.

In regard to the BM, we observed no differences in the recovery of femoral CFU-GM between young and aged mice treated with 5-FU; these findings are consistent with previous reports.[13,14] In contrast, the recovery process for femoral CFU-mast cells was quite different between young and aged mice. Namely, young mice showed higher recovery (122% of steady state) on day 21 after 5-FU treatment, whereas aged mice showed lower recovery (64% of steady state) on day 14 after 5-FU treatment. This difference in the recovery process in CFU-mast number between young and aged mice after 5-FU treatment is comparable with the difference in the recovery process in the CFU-preB number between young and aged mice after 5-FU treatment.[13,14]

In the BM of young SAMP1 mice, the expression of the SCF gene is one-thirtieth that of TGF-β during the steady state (Figure 3a). After 5-FU treatment, the SCF mRNA levels in the BM markedly increased approaching TGF-β mRNA levels and remained high for nearly a week (Figure 3a). Furthermore, the SCF/TGF-β ratio immediately peaked and remained high for nearly a week (Figure 3c). The number of CFU-mast cells in the S-phase after cytoablation with 5-FU is a good indicator of the balance between the positive and negative regulation of mast-cell development by stromal cells.[19] The number of femoral CFU-mast cells in the S-phase in young mice after 5-FU treatment recovered from its nadir and exceeded the pretreatment levels until day 28 (Figure 5a). These data are compatible with data from regular strains of mice such as C3H/HeN.[19] Thus, these data indicate that the regulation of mast-cell development is dominated by negative signals during the steady state and that the negative dominant regulation is converted to positive dominant regulation under perturbed conditions induced by 5-FU.

The oscillation of the gene expression levels of SCF and the SCF/TGF-β ratio in the BM of aged mice is different from that in young mice (Figure 3a versus b, Figure 3c versus d). In contrast to young mice, the gene expression levels of SCF and the SCF/TGF-β ratio in aged mice after 5-FU were not kept high (Figures 3b and d). Furthermore, the number of femoral CFU-mast cells in the S-phase of aged mice after 5-FU treatment showed a lower recovery (47% of steady state), whereas the number of femoral CFU-mast cells in the S-phase in young mice showed a higher recovery (205% of steady state) (Figure 5a). Taken together, these data suggest that senescent stromal cells in aged 5-FU-treated mice could not continuously maintain the positive dominant regulation for mast-cell development, resulting in impaired mast-cell regeneration in aged mice.

When young and aged mice were treated with 5-FU, the recovery processes in the number of splenic CFU-GM in young and aged mice was comparable. Namely, young and aged mice show a high recovery (young mice, 668% of steady-state levels; aged mice, 506% of steady-state levels). In contrast, the recovery process of the number of splenic CFU-mast cells was quite different between young and aged mice. Namely, the young mice showed a higher recovery (200% of steady state), whereas aged mice showed a lower recovery (61% of steady state).

In the case of young mice, although the gene expression levels of SCF and the SCF/TGF-β ratio in the spleens of young mice after 5-FU treatment showed limited oscillations, the SCF/TGF-β ratio after day 7 was continuously increased to two-fold the pretreatment levels (Figures 4a and c). The number of splenic CFU-mast cells in the S-phase of young mice after 5-FU treatment recovered from nadir and overshot to 143% of pretreatment levels (Figure 5b). In contrast to the BM, the regulation of mast-cell development in the spleen does not change significantly even after 5-FU treatment, which indicates that the positive dominant regulation of mast-cell development of the spleen after 5-FU treatment is limited. The spleen may mainly act as a reservoir for mast-cell progenitor cells, whereas the BM may act as a source of mast-cell progenitors via induction of hematopoietic stem cell differentiation into mast cells.[19,38,39] The results of the spleen of young SAMP1 mice are compatible with previous reports.

In the case of aged mice, when compared with young mice, the gene expression levels for SCF and the SCF/TGF-β ratio in the spleen of aged mice after 5-FU treatment are markedly decreased during the first seven days, and the SCF/TGF-β ratio after day 7 was not kept high (Figure 4a versus b, Figure 4c versus d). Furthermore the recovery of the number of CFU-mast cells in the S-phase in the spleen of aged mice is limited (35% of steady state), whereas young mice showed higher recovery (143% of steady state) (Figure 5b). These data suggest that the lower recovery of the number of splenic CFU-mast cells in aged mice may be not only due to the decrease of the number of CFU-mast cells that migrated from the BM but also due to age-related impairment of splenic stromal cells.

The stromal element is quantitated by CFU-F assay. In the steady state, the number of CFU-F in the BM of aged SAMP1 mice is two fold that of young SAMP1 mice, which is identical with our previous report (Figure 6).[5] When young and aged mice were treated with 5-FU, the extent of decrease of CFU-F number was smaller in aged mice (35% of steady state) than in young mice (10% of steady state) (Figure 6). These data indicate that CFU-F in aged mice is less sensitive to 5-FU compared with young mice which may reflect that the quality of CFU-F changes with aging. Stolzing and Scutt[40] reported that the reduced ability to maintain mesenchymal tissue homeostasis in aged mammals is not purely due to a decline in progenitor cell number but also to a loss of progenitor functionality. Taken together, age-related stromal cell impairment in SAMP1 mice may be due to a loss of functionality in mesenchymal progenitor cells and their progenies.

Cumulative evidence indicates that aging exerts significant effects on all cells of the innate immune system, which is a contributing factor to aberrant outcomes after injury or infection and to the development of many diseases observed in the elderly.[2] Mast cells also play a key role in the innate immune system. Several kinds of cytokines produced by stromal cell other than SCF and TGF-β are also involved in mast cell development. However, since SCF and TGF-β are representative cytokines to regulate mast cell development, the age-related changes of oscillation of SCF/TGF-β ratio which indicate the balance of positive and negative signals for mast cell development seem to reflect age-related functional impairment of stromal cells. Therefore, our results indicated that age-related functional impairment of stromal cells deteriorated mast-cell development as observed in B lymphopoiesis. Thus, age-related deterioration of mast-cell development due to senescent stromal cells is considered to contribute, in part, to immunosenescence.

Author contributions: All authors participated in the design, interpretation of studies, analysis of the data and review of manuscript. IT, TH, YH, JK and SA conducted the experiments, and IT, TI and SA wrote the manuscript.

ACKNOWLEDGEMENTS

We thank Drs Kiyoshi Sekita and Yukio Kodama, Erika Tachihara and Minako Kenjoh for assistance in maintaining the SAMP1 mice in the experimental animal facilities, and we thank Sonoko Araki and Miyuki Yuda for their technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research (C) and Grant-in Aid for Young Scientist (B) from the Japan Society for the Promotion of Sciences.

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(Received May 10, 2012, Accepted August 9, 2012)