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Long-term administration of pDC-Stimulative *Lactococcus lactis* strain decelerates senescence and prolongs the lifespan of mice

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ABSTRACT

The decline in immune function caused by aging increases the risk of infectious diseases, tumorigeneses and chronic inflammation, resulting in accelerating senescence. We previously reported a lactic acid bacteria, *Lactococcus lactis* strain Plasma (synonym of *Lactococcus lactis* subsp. *lactis* JCM 5805, Lc-Plasma), that stimulates plasmacytoid dendritic cells (pDCs), which play a crucial role in phylaxis from viral infection. In this study, we investigated the anti-aging effects of long-term oral administration of Lc-Plasma in a senescence-accelerated mouse strain, SAMP6. Mice given Lc-Plasma showed a significant improvement in survival rate at 82 weeks and a decreased senescence score as compared with control mice throughout this study. Anatomic analysis at 82 weeks revealed that the frequency of altered hepatocellular foci was significantly lower, and the incidence of other pathological findings in the liver and lungs tended to be lower in Lc-Plasma mice than in control mice. Transcription level of the *IL-1β* gene in lungs also tended to be lower in Lc-Plasma mice. Furthermore, the thinning of skin and age-related decrease in muscle mass were also significantly suppressed in the Lc-Plasma group as compared with the control group. Consistent with these phenotypic features, pDCs activity was significantly higher in Lc-Plasma mice than in control mice. In conclusion, long-term administration of Lc-Plasma can decelerate senescence and prolong lifespan via maintenance of the immune system due to activation of pDCs.

1. Introduction

Immune function decreases during aging, resulting in increased susceptibility to infectious diseases and risk of cancer [1,2]. The decline in immune function induces chronic inflammation, resulting in accelerating senescence [3]. A number of studies reporting age-related dysfunctions in adaptive immunity have been published. Decreased thymic T cell generation and disruption of homeostatic T cell proliferation have been reported [4,5]. The number of B cells decreases due to the loss of early precursors [6], and immunoglobulin class switching is depressed [7,8]. Age-related dysfunction of innate immune cells — namely, macrophages, NK cells and dendritic cells (DCs) — has also been reported [9,10]. In addition, DC tumor antigen presentation has been shown to be defective in mDCs from aged mice, and a selective decrease in DC-SIGN has been observed [11].

Plasmacytoid dendritic cells (pDCs) are a crucial subset of cells in anti-viral immunity that act as proficient producers of type I IFN [12,13]. Furthermore, pDCs and pDC-derived type I IFNs act as regulators in both adaptive and innate immunity by controlling various immune factors, such as T cells [14–17], B cells [18,19], and NK cells [20]. Any deterioration in the function of pDCs seriously affects the whole immune system, because they are responsible for not only innate immune functions such as phagocytosis and cytokine secretion in response to antigens, but also acquired immunity by means of antigen presentation and priming T cells and B cells [21,22].

We previously discovered a unique type of lactic acid bacteria (LAB), *Lactococcus lactis* strain Plasma (Lc-Plasma), which stimulates murine pDCs in vitro and in vivo [23]. Lc-Plasma is a synonym of *Lactococcus lactis* subsp. *lactis* JCM 5805. The active component responsible for pDC activation is demonstrated to be DNA. We revealed that IFN-α production by pDC was induced by Lc-Plasma derived DNA, in addition, it was depending on the TLR9-MyD88 pathway [23]. Animal experiments using a parainfluenza-infection model revealed that oral administration of Lc-Plasma markedly increased the survival rate against infection and enhanced lung anti-viral immune responses through the activation of intestinal pDCs [24]. Lc-Plasma could also

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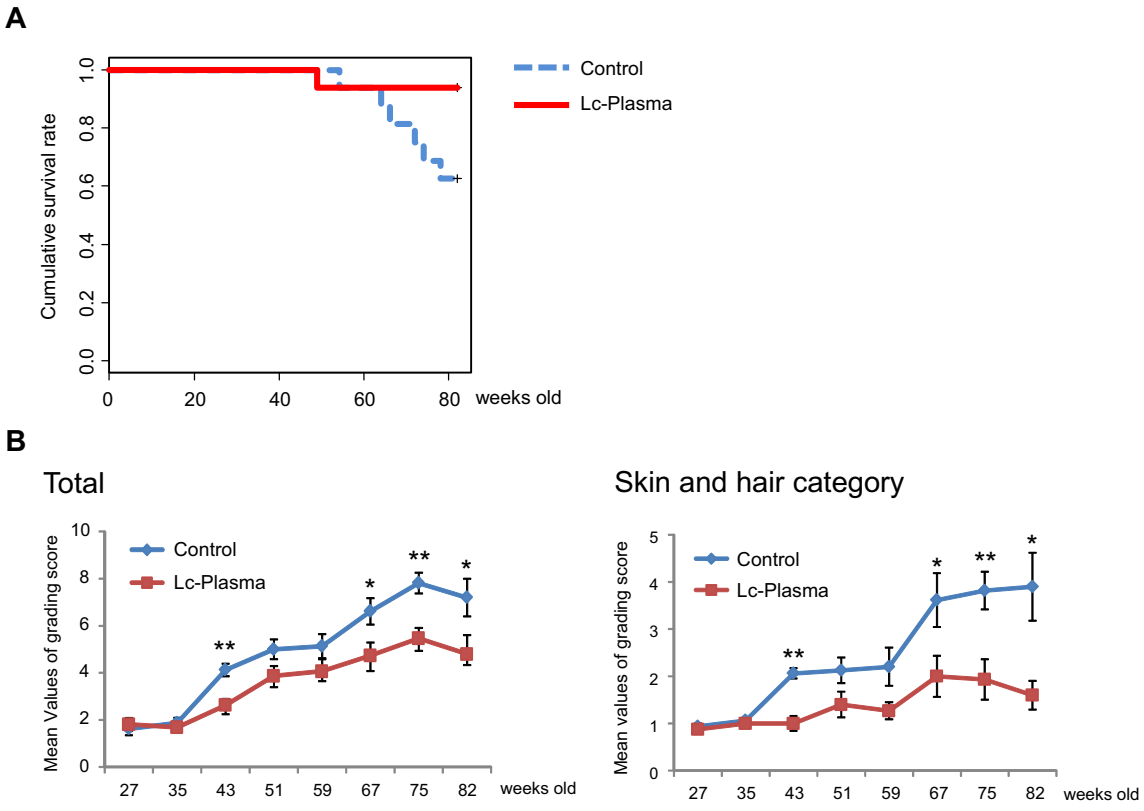

Fig. 1. Comparison of the cumulative survival rate and chronological changes in the degree of senescence.
The cumulative survival rate was evaluated at age 82 weeks (A). The control (dot-line) and Lc-Plasma (line) groups each consisted of 16 mice. $P < 0.05$ (Log-Rank test).
The degree of senescence was recorded once every 8 weeks (B). Total scores and scores in the skin and hair category were shown. The control and Lc-Plasma groups each consisted of 16 mice. The mean value of grading score was compared between the two groups. Bar graph shows the mean ± SE grading score. $^{*}P < 0.05$, $^{**}P < 0.01$ (Mann-Whitney *U* test).

activate human pDCs in vitro and in vivo [25]. In a human clinical study, oral administration of Lc-Plasma for 10 weeks decreased the pathogenesis of an influenza-like illness via enhancement of an IFN-α response to influenza virus [26].

LAB are widely accepted as safe and can be easily incorporated in the daily diet; therefore, our research has focused on the effects of lifelong daily administration of Lc-Plasma on phenotypes caused by aging. Senescence-accelerated mouse (SAM) is an animal model of accelerated senescence and senescence-associated disorders that presents age-related phenotypes closely mimicking human aging dysfunctions [27,28]. SAMP6 strain is known to have some aging features, such as senile osteoporosis, secondary amyloidosis, and colitis; on the other hand, dysfunction of the immune system, as in SAMP1, SAMP2 and SAMP8 strains, has not been reported [29]. Therefore, SAMP6 might be useful for evaluating the natural aging dysfunction of the immune system.

Some LAB and their products have been reported to be effective in suppressing various phenotypes and dysfunctions of the immune system in SAM. For example, oral administration of *Lactococcus lactis* subsp. *cremoris* H61 to aged SAMP6 for 5 months was associated with a reduction of bone density loss [30]. Administration of *Lactobacillus gasseri* TMC0356 to SAMP1 for 4 and 8 weeks indicated the potential to upregulate cell-mediated immunity [31]. Administration of LAB-fermented milk for 4 months had a preventive effect against inflammatory bowel disease in SAMP1/Yit mice [32]. However, the effect of long-term administration of LAB and the relationship between immune function and aging remain unknown.

Here, we investigated the anti-aging effects of lifelong administration of Lc-Plasma in female SAMP6 mice. Age-related phenotypes were improved by the suppression of inflammation; furthermore, lifespan was prolonged markedly.

2. Materials and methods

2.1. Preparation of LAB

Lactococcus lactis subsp. *lactis* JCM 5805 (Lc-Plasma) were purchased from the Japan Collection of Microorganisms. LAB strains were grown at 30 °C for 48 h in M17 broth (Oxoid Ltd.) in accordance with the manufacturer's instructions, washed twice with sterile distilled water, and heat-killed at 100 °C for 30 min.

2.2. Mice

Five-weeks-old SAMP6 female mice were purchased from Japan SLC, Inc. We have confirmed the effect to activate pDCs by administration of Lc-Plasma on female mice [22], therefore we chose female mice in order to compare with previous study. Mice were housed individually in a cage. Temperature was maintained at 23 ± 2 °C, lighting was set at a 12 h / 12 h light/dark cycle, and humidity was fixed at 60 ± 15%. Animal procedures and experiments were approved by the Laboratory Animal Care Committee of Central Laboratories for Key Technologies, Kirin Co., Ltd. (approval ID YO12-00050).

Mice were divided into two groups based on body weight. Lc-Plasma administration was started from 7 weeks of age. Control group mice ($n = 20$) were fed AIN93G (Oriental Yeast, Tokyo, Japan), and Lc-Plasma group mice ($n = 20$) were fed AIN93G containing 1 mg of heat-killed Lc-Plasma per day per mouse until age 12 weeks. At 12 weeks, the

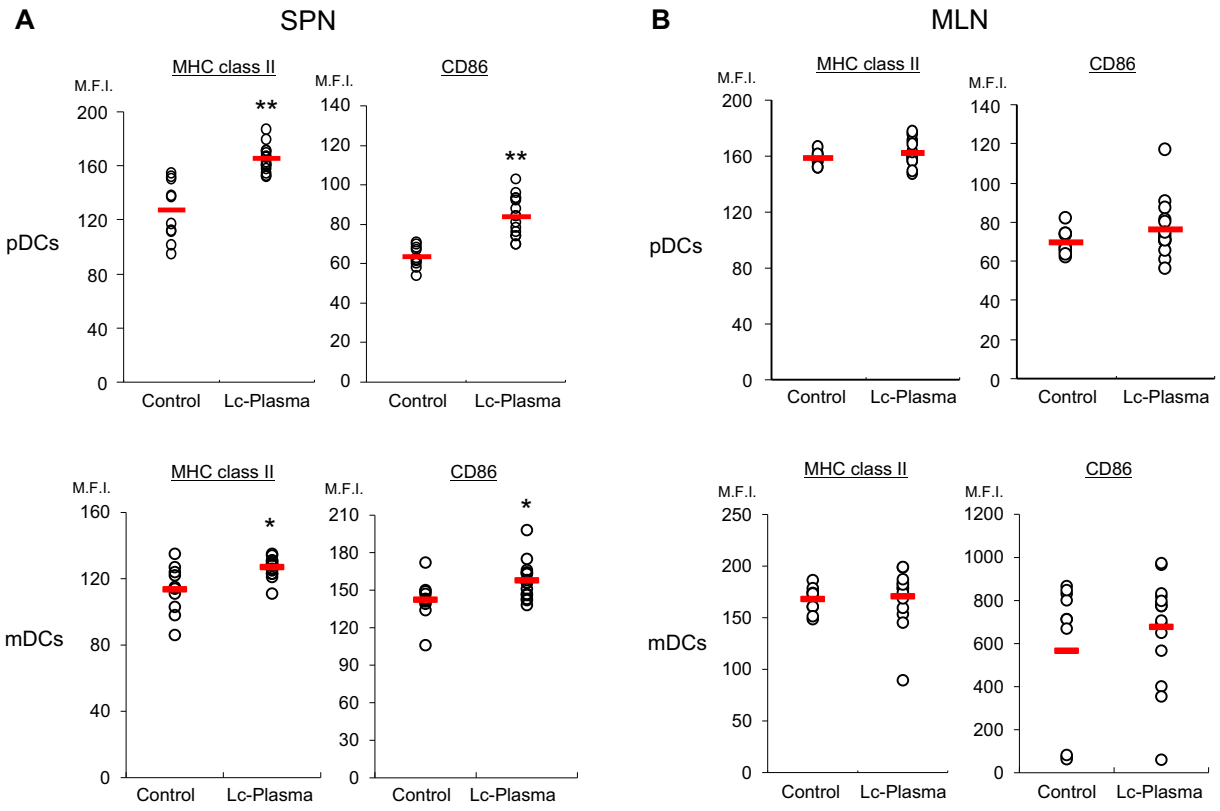

Fig. 2. Comparison of pDC and mDC activity.
Shown is the effect of long-term administration of Lc-Plasma on the expression of activation markers on pDCs and mDCs in the SPN (A) and MLN (B). The expression levels of MHC class II and CD86 are shown as median fluorescence intensities (M.F.I.) in the left and right panels, respectively. Short lines represent the mean values. The control and Lc-Plasma groups consisted of 10 mice and 15 mice, respectively. $*P < 0.05$, $**P < 0.01$ (Student's *t*-test).

base feed of both groups was changed from AIN93G to AIN93M in accordance with the recommended method [33]. Net body weights were measured once every 4 weeks from 7 weeks until 82 weeks of age. Blood samples were collected once every 8 weeks. Four mice in each group died due to the blood collection procedure during the study and were excluded from the analysis; as a result, data from 16 mice in each group were analyzed.

2.3. Survival analysis

The number of survivors was recorded every day. Survival rate until 82 weeks of age was evaluated by using a log-rank test. The endpoint was defined before the study began as the point where the number of survivors dropped to 10 or fewer in either group.

2.4. Evaluation of senescence

Evaluation of the degree of senescence in SAM was performed by using the grading score system previously reported [28]. In short, seven items divided into three categories were recorded once every 8 weeks as follows: 1) behavior category (reactivity and passivity); 2) skin and hair category (glossiness, coarseness, hair loss, and ulcer); and 3) spine category (lordokyphosis).

2.5. Pathologic analysis

Mice were sacrificed at 82 weeks of age, after administration of Lc-Plasma for 75 weeks. Spleen (SPN), mesenteric lymph node (MLN), lungs, liver, kidneys, dorsal skin, leg muscle, and blood were collected from the control ($n = 10$) and Lc-Plasma ($n = 15$) groups.

Lung, liver, and kidney were fixed by using 10% formalin. Pathologic tissue analyses were evaluated by the Public Interest Incorporated Foundation BioSafety Research Center (Japan). Samples were embedded in paraffin and thinly sectioned. After de-paraffinization, sections were counterstained with Mayer's hematoxylin solution (Muto Pure Chemicals Co., Ltd., Merck, mixture of equal amounts) and eosin solution, dehydrated in tissue dehydration solution (Wako), cleared in xylene, and covered with a coverslip using HSR solution (Sysmex). Samples containing substances unable to be unidentified by HE staining were analyzed by periodic acid-schiff (PAS), periodic acid/methenamine-silver (PAM), Masson trichrome stain, and Congo red staining. Gross lesions were also recorded. Histopathological analysis was performed by using a pathological examination system (PATHOTOX). Histopathological findings were analyzed for 11 items in the lung category, 23 items in the liver category, and 9 items in the kidney category.

2.6. Cell preparation and FACS analysis

SPN cells and MLN cells were used to evaluate the activity of innate immunity, that is, the activity of DCs including both pDCs and mDCs, by FACS analysis. Cells were minced in Mg^{2+} and Ca^{2+} free Hank's Balanced Salt Solution and digested with 1 mg/ml of collagenase (Sigma) and 0.2 mg/ml of DNase I for 20 min at 37 °C. EDTA was added to give a final concentration of 30 mM and the mixture was incubated for 10 min at room temperature. Tissue lysates were filtered through a 70 μm nylon cell strainer, layered onto 15% Histodenz (Sigma) in RPMI 1640 containing 10% FCS, and centrifuged at 450 ×*g* for 20 min without braking. Low-density fractions at the interface were collected and washed. The cells were stained with a fluorescent dye conjugated to an antibody as follows: MHC class II-FITC (M5/114.15.2) (eBiosciences), CD86-PE (GL1) (BD Pharmingen), Siglec-H-APC

Table 1
Pathologic analysis of lung, liver and kidney in the two groups.

Organ	Pathological finding	Number of mice		P value (χ^2 test)	
		Control $n = 10$	Lc-Plasma $n = 15$		
Lung	Hemorrhage	6	9	1.000	
	Thrombus	0	1	0.405	
	Accumulation of foamy cells	1	0	0.211	
	Appearance of giant nucleus	2	0	0.071	†
	Eosinophilic change	10	15	–	
	Metaplasia, osseous	0	1	0.405	
	Metaplasia, squamous cell	0	0	–	
	Mineralization	1	0	0.211	
	Aggregation, macrophage	2	0	0.071	†
	Neutrophilia	10	15	–	
	Hyperplasia, lymphoid tissue	0	0	–	
Liver	Peliosis	1	0	0.211	
	Anisonucleosis, hepatocyte	4	7	0.742	
	Atrophy, hepatocyte	0	0	–	
	Cyst	1	1	0.763	
	Dilatation, sinusoid	1	0	0.211	
	Dilatation, bile duct	3	2	0.307	
	Hyaline droplet, hepatocyte	0	0	–	
	Hyalinosis, bile duct	1	0	0.211	
	Hypertrophy, sinusoidal cell	1	0	0.211	
	Necrosis, hepatocyte	2	4	0.702	
	Vacuolation, hepatocyte	1	1	0.763	
	Abscess	2	0	0.071	†
	Bacterial infection	2	0	0.071	†
	Aggregation, macrophage	3	4	0.856	
	Cholangitis	1	0	0.211	
	Infiltration, inflammatory cell	4	9	0.327	
	Extramedullary hematopoiesis	3	1	0.119	
	Hypertrophy, bile duct epithelial cell	0	1	0.405	
	Increase in mitosis, hepatocyte	1	2	0.802	
	Altered hepatocellular foci	5	2	0.045	*
	Hyperplasia, bile duct	2	1	0.315	
	(Adenoma, hepatocellular)	1	2	0.802	
	Hemangiosarcoma	2	1	0.315	
	Sarcoma, histiocytic	0	1	0.405	
Kidney	Cast, hyaline	3	7	0.405	
	Cyst	1	1	0.763	
	Deposit, amyloid, glomerulus	0	1	0.405	
	Eosinophilic amorphous substance, glomerulus	5	7	0.87	
	Glomerulopathy, mesangioproliferative glomerulonephritis	4	4	0.484	
	Hyaline droplet, proximal tubule	1	1	0.763	
	Regeneration, tubule	7	10	0.861	
	Infiltration, inflammatory cell	1	1	0.763	
	Extramedullary hematopoiesis	0	1	0.405	

Pathologic observations of lung, liver, and kidney from the two groups are shown. The number of mice with abnormalities related to 11 items in lung, 23 items in liver, and 9 items in kidney were counted. $*P < 0.05$, $\dagger P < 0.1$ (χ^2 test).

(551.3D3) (Miltenyi Biotec), CD11c-PE-Cy7 (N418) (eBiosciences), CD11b-APC-Cy7 (M1 / 70) (BD Pharmingen), and CD8-PerCP (53–6.7) (BD Biosciences). After staining, cells were washed twice with FACS buffer (0.5% BSA in PBS buffer) and suspended in 4% paraformaldehyde (Wako) for FACS analysis. Data were collected by a FACS Canto II instrument (BD Biosciences) and analyzed by FCS Express software (De Novo Software). $CD11c^+$ $Siglec\text{-}H^+$ cells and $CD11c^+$ $CD11b^+$ cells were defined as pDCs and myeloid DCs (mDCs), respectively. The expression levels of cell surface activation markers (MHC class II and CD86) on pDCs and mDCs were measured.

2.7. Gene expression analysis

Total RNA was extracted from the lung and liver of both groups using RNeasy Kit (Qiagen). cDNA was prepared by using an iScript cDNA synthesis kit (BioRad), in accordance with the manufacturer's protocol. qRT-PCR was performed using SYBR Premix EX Tag (TaKaRa) using a LightCycler 480 (Roche). GAPDH was used as the reference gene. The primer sequences were as follows: *Gapdh* 5′-AACGACCCCT TCATTGAC-3′ and 5′-TCCACGACATACTCAGCAC-3′; *Il-1β* 5′-TTGACG-GACCCCAAAABATG-3′ and 5′-TGGACAGCCCAGGTCAAAG-3′; *TNFα* 5′-GATCGGTCCCCAAAGGGATG-3′ and 5′-TGAGGGTCTGGGCCATA GAA-3′; *SAA1* 5′-ATGAAGGAAGCTAACTGGAAAAACTC-3′ and 5′-TCC TCCTCAAGCAGTTACTACTGCAA-3′; *SAA2* 5′-ATGAAGGAAGCTGGCT GGAAAGATGG-3′ and 5′-TCCTCCTCAAGCAGTTACTACTGCTG-3′; *SAA3* 5′-GCCACCATGAAGCCTTCCATTGCCATCATT-3′ and 5′-TCAGT ATCTTTTAGGCAGGCCAGCAG-3′.

2.8. Evaluation of skin thickness

Dorsal skins collected from mice were fixed by using 10% formalin. After dehydration, samples were embedded by paraffin, thinly sectioned (4 μm), and stained by HE using the conventional method. Cross-sections were selected from four plates per sample and one microscopic field (100× magnification) per plate was photographed. Ten observation points were selected from one plate at random. The average thickness of the epidermis from one sample was calculated from 40 points (10 points × 4 plates).

2.9. Evaluation of muscle weight

Soleus muscle, gastrocnemius muscle, tibialis anterior muscle, and extensor digitorum longus muscle were collected from both legs. The average weight of both legs per body weight was calculated.

2.10. Statistical analysis

Statistical differences between two groups were determined by using an unpaired, two-tailed Student's *t*-test with significance set at $P < 0.05$. For survival studies, a Log-Rank (Mantel-Cox) test was used. Senescence scores were evaluated by using the Mann-Whitney *U* test. Pathological analysis of lung, liver, and kidney was evaluated by using a χ^2 test.

3. Results

3.1. Long-term administration of Lc-Plasma decelerates senescence and prolongs lifespan

At 82 weeks of age, the survival rate was 62.5% (10/16) in the control group, and 93.75% (15/16) in Lc-Plasma group (Fig. 1A). The cumulative survival rate was significantly higher in the Lc-Plasma group than in the control group ($P < 0.05$).

Senescence scores were evaluated once every 8 weeks from 27 weeks until 82 weeks of age, as described in Materials and Methods. The total scores were lower in the Lc-Plasma group than in the control group throughout the study; in particular, there were significant differences at 43, 67, 75, and 82 weeks (Fig. 1B). Regarding the sub-categories, scores in the skin and hair category were significantly lower in the Lc-Plasma group (Fig. 1B); however, there were no significant differences between the two groups in the behavior category and spine category (data not shown). These results suggest that daily administration of Lc-Plasma prolongs lifespan and decelerates senescence, with the greatest effect on scores in the skin and hair category.

3.2. Long-term administration of Lc-Plasma activates pDCs and mDCs in aged mice

We previously reported that administration of Lc-Plasma to young mice for 2 weeks activated pDCs and mDCs in mesenteric lymph node (MLN), but not in spleen (SPN) [23]. In this study, we evaluated the

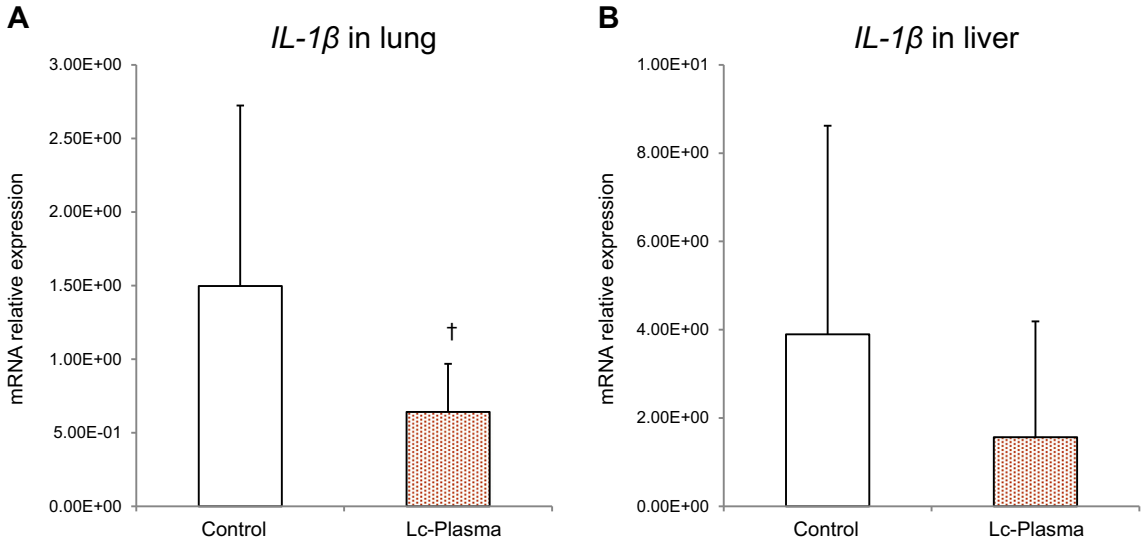

Fig. 3. Comparison of the transcription level of *IL-1β* in liver and lungs.
qRT-PCR was performed using cDNA prepared from total RNA extracted from lung (A) and liver (B). *GAPDH* was used as the reference gene. The control and Lc-Plasma groups consisted of 10 mice and 15 mice, respectively. The mean ± SD of the tissues in each group is shown. $^{\dagger}P < 0.1$ (Student's *t*-test).

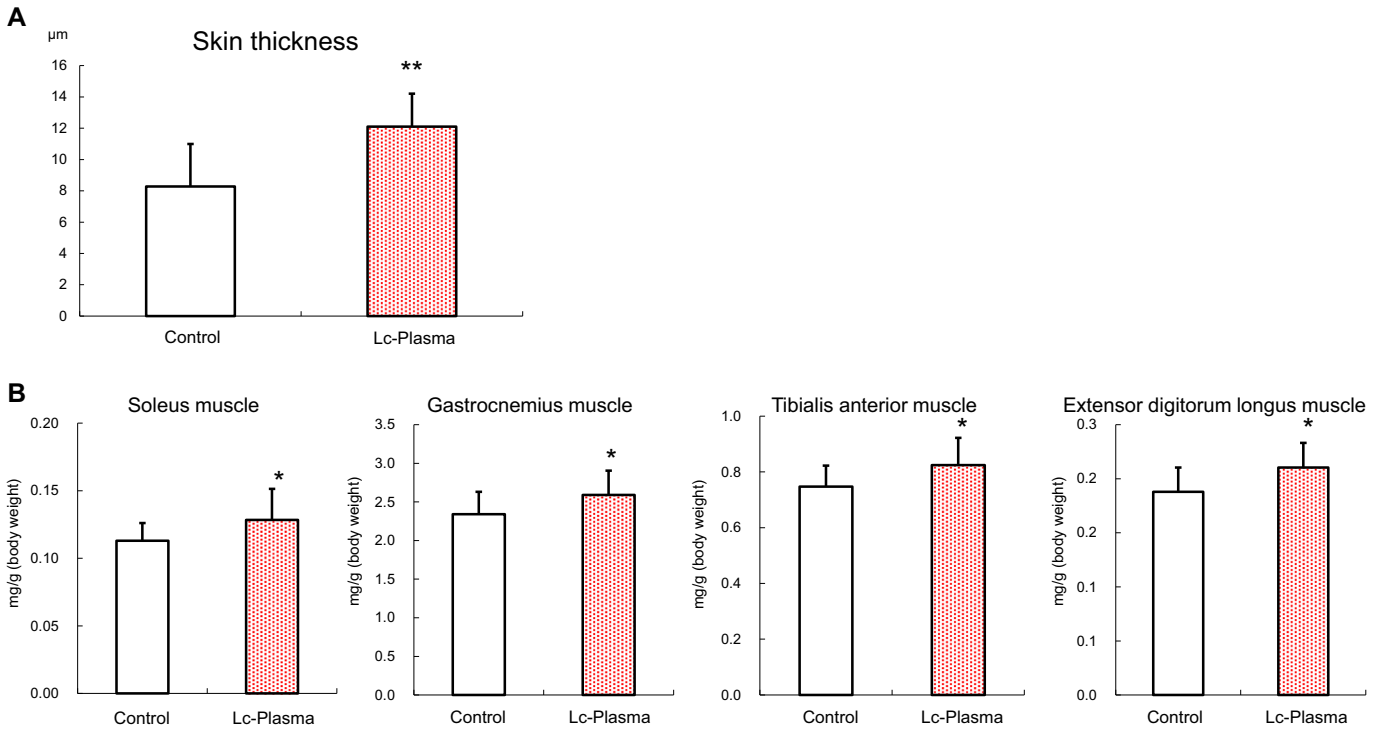

Fig. 4. Comparison of skin thickness and muscle weight.
Skin thickness was evaluated at age 82 weeks (A). The control (open columns) and Lc-Plasma (dot columns) groups consisted of 10 mice and 15 mice, respectively. The mean ± SD of the tissues in each group is shown. $^{**}P < 0.01$ (Student's *t*-test).
Muscle weight was evaluated at age 82 weeks (B). Control (open columns) and Lc-Plasma (dot columns) groups consisted of 10 mice and 15 mice, respectively. The mean ± SD of the tissues in each group is shown. $^{*}P < 0.05$ (Student's *t*-test).

effects of lifelong administration of Lc-Plasma on both splenic and intestinal pDCs and mDCs. We examined the activation of pDC and mDC at 82 weeks of age, after administration of Lc-Plasma for 75 weeks. As shown in Fig. 2A, the expression level of MHC class II and CD86 on splenic pDCs were significantly higher in the Lc-Plasma group than in the control group ($P < 0.01$). Furthermore, activation markers on splenic mDCs were higher in the Lc-Plasma group than in the control group ($P < 0.05$) (Fig. 2A). Contrary to the data obtained after 2 weeks of administration of Lc-Plasma in young mice [23], the expression of activation markers on pDCs and mDCs in MLN did not differ significantly between the two groups (Fig. 2B). These results indicate that lifelong administration of Lc-Plasma might be able to inhibit the age-related decrease in activity of systemic pDCs and mDCs.

3.3. Effect of long-term administration of Lc-Plasma on pathogenesis and inflammation

SAMP6 mice are reported to die mainly because of pneumonia,

abscess formation and contracted kidneys [29]; therefore, we performed a pathological examination of the lungs, liver, and kidneys at 82 weeks of age (Table 1). The frequency of altered hepatocellular foci was significantly lower in the Lc-Plasma group than in the control group ($P < 0.05$), and the onset of abscesses and bacterial infection in the liver, and the appearance of giant nuclei and aggregation of macrophages in the lungs tended to be lower in the Lc-Plasma group ($P < 0.1$).

The various anatomical differences in the liver and lungs between the two groups prompted us to evaluate gene transcription levels of *IL-1β*, which is described as a central mediator in inflammation [34]. qRT-PCR analysis showed that the transcription level of *IL-1β* in lungs tended to be lower in the Lc-Plasma group than in the control group ($P = 0.057$) (Fig. 3A). On the other hand, there was no significant difference in the transcription level of *IL-1β* in liver between groups (Fig. 3B). In addition, we also evaluated the transcription level of *SAA1*, *SAA2*, *SAA3*, and *TNF-α*, which are also well-known biomarkers of aging and chronic inflammation, however there were not significant differences. The transcriptions of *SAA1* and *SAA2* in liver were confirmed in only 2 mice in the control groups (data not shown).

These results suggest that lifelong administration of Lc-Plasma might suppress the onset of pathological aberrations by regulating inflammatory gene expression.

3.4. Effect of long-term administration of Lc-Plasma on skin and muscle cell senescence

As mentioned above, the scores in the skin and hair category were significantly improved in the Lc-Plasma group. Because it has been reported that the epidermis becomes thin during the aging process [35,36], we measured dorsal epidermis thickness at 82 weeks. The data showed that dorsal epidermis was significantly thicker in the Lc-Plasma group than in the control group ($P < 0.01$) (Fig. 4A).

We also measured the weights of the soleus, gastrocnemius, tibialis anterior and extensor digitorum longus muscles in both legs: all four muscles were significantly heavier in the Lc-Plasma group than in the control group ($P < 0.05$) (Fig. 4B).

Taken together, these results indicate that lifelong administration of Lc-Plasma might suppress the acceleration of senescence in skin and muscle.

4. Discussion

We previously reported that administration of Lc-Plasma activates pDCs, resulting in protection from viral infection in both mice and humans [23–26]. In this study, we found that administration of Lc-Plasma is also effective in decelerating senescence. Furthermore, our data indicated the significant effect of prolonging lifespan. At 82 weeks of age, the survival rate of SAMP6 mice fed Lc-Plasma was higher than that of the control mice. Six out of 16 mice died in the control group, but only one out of 16 mice died in the Lc-Plasma group. The activities of pDCs and mDCs in SPN were higher in the Lc-Plasma group than in the control group; however, the activities of pDCs and mDCs in MLN did not differ between the two groups. We previously reported that administration of Lc-Plasma for 2 weeks stimulated DC activity in MLN, but not in SPN [23]. These results suggest that Lc-Plasma might have different effects, depending on the terms of administration. On the one hand, short-term administration of Lc-Plasma may activate local immune cells transiently and contribute to a marked increase in phylaxis from infection; on the other hand, long-term administration of Lc-Plasma may inhibit the continuous decline of systemic immunity system and contribute to decelerating senescence phenotypes and prolonging lifespan.

One of the functions of pDCs, type I IFN production, is reported to be decreased in aged C57BL/6 mice [37]. DC tumor antigen presentation has also been found to be defective in mDCs from aged mice, and a selective decrease in DC-SIGN has been observed [11]. These defects in DC function may lead to a risk of infectious diseases. In this study, the activity of pDCs and mDCs, representing the immune subsets against viruses and bacteria, respectively, was maintained at a higher level until the end of life in the Lc-Plasma group as compared with the control group. Therefore, the risk of daily opportunistic infection might be reduced throughout lifespan by Lc-Plasma administration. Indeed, bacterial infection in the liver was found specifically in only the control group (Table 1), which is considered to be the effect of mDC activation.

Increased frequency of infection can lead to an accumulation of damage-associated molecular patterns (DAMPs), which activate NLRP3 (inflamasome dependent caspase-1) and cause increased production of pro-inflammatory cytokines [38,39]. In this study, the transcription level of *IL-1β* in lungs tended to be suppressed in the Lc-Plasma group. The transcriptions of *SAA1* and *SAA2* in liver were confirmed in only the control group. IL-1β is known as a central mediator in inflammation [34], and *SAA* gene family was reported to be highly expressed during acute inflammation and chronic inflammatory diseases [40]. In pathological observations, the frequency of altered hepatocellular foci was significantly improved in the Lc-Plasma group. In addition, the onset of abscesses in the liver, and the appearance of giant nuclei and aggregation of macrophages in the lungs were found in only the control group (Table 1). These results suggest that long-term administration of Lc-Plasma may inhibit the accumulation of chronic inflammation through the suppression of daily infection. We speculate that the main reason why there was not statistic difference between two groups regarding with those parameters is six out of 16 mice were died in control group before the endpoint and we could not collect those data of control group. Furthermore, we previously reported that in vitro Lc-Plasma stimulation of pDCs induced ICOS-L and PD-L1 and resulted in the induction of $CD4^+CD25^+Foxp3^+$ Treg cells [23], suggesting that Lc-Plasma administration might contribute to suppression of the inflammatory response via the generation of DCs. We have evaluated the number of pDC and mDC, although there was no significant difference between two groups (data not shown). Therefore, we consider that long-term administration of Lc-Plasma did not affect the number of DCs. Also, concentrations of type I IFN in blood serum at 82 weeks were not detected in both groups. However, it is reported that an excessive increase of IFN production lead to chronic inflammation causing acceleration of senescence, therefore we think these results do not dismiss the notion of anti-aging effect. More importantly, long-term administration of Lc-Plasma could inhibit the decline of co-stimulatory molecules or MHC on DCs which lead to preservation of appropriate tuning of acquired immunity and contribute to improvement of reactivity to virus and other immune systems.

We also evaluated the effects of Lc-Plasma on tissue damage associated with aging in this study. As a result, age-related skin thinning and reduced muscle weight were significantly suppressed in mice fed Lc-Plasma, which might have contributed to the marked improvement of senescence scores in the Lc-Plasma group. Recently, an interesting new role of pDCs in sensing tissue and promoting wound healing in skin has been reported, and the activation of pDCs to produce type I IFN was found to be dependent on endosomal TLR7 and TLR9 [41]. This suggests that pDCs can recognize nucleic acids released from organs degraded by aging and can heal the damage. Type I IFNs would temporarily induce early inflammatory factors for wound healing, which might contribute to the suppression of chronic inflammation. We also hypothesize that the anti-aging effect of pDCs is due to the suppression of chronic inflammation through wound healing. Various conventional LABs are reported to activate mDC or macrophages, however LAB that activate not only mDC but also pDC is not reported elsewhere. The effect via pDC activation such as wound healing might contribute in long-term administration of Lc-Plasma. To clarify the precise effect of pDC activation in wound healing, it is needed to be compared with other conventional LAB strains that activate only macrophages or mDC in the future.

In conclusion, we have reported new effects of Lc-Plasma in this study. Long-term administration of Lc-Plasma decelerated senescence and prolonged the lifespan of mice markedly. Lc-Plasma is a rare LAB that can activate not only mDC but also pDC. We hypothesize that such total DCs activation in aged mice contributes to suppression of abnormal inflammation by the effects of "infection prevention", "Treg cell induction", and "wound healing", resulting in decelerating senescence and prolonging lifespan. We consider that the abnormal inflammation does not gradually rise with age, but rather occurs due to a declining function of the upstream immune system. However it's still unclear whether other effects such as suppression of pathological onset and inflammation, or suppression of skin and muscle aging are caused by direct mechanism via DCs activation. Studies exploring the detailed mechanisms underlying the relationship between Lc-Plasma administration and aging phenotypes will be required.

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Author contributions

D.F., T.S. conceived and designed the experiments, T.S., K.J., K.O. and others performed the experiments, T.S., K.J., K.O. and others analyzed the data, T.S., K.J., D.F. wrote the manuscript.

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Competing interests

The authors declare no competing interests.

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