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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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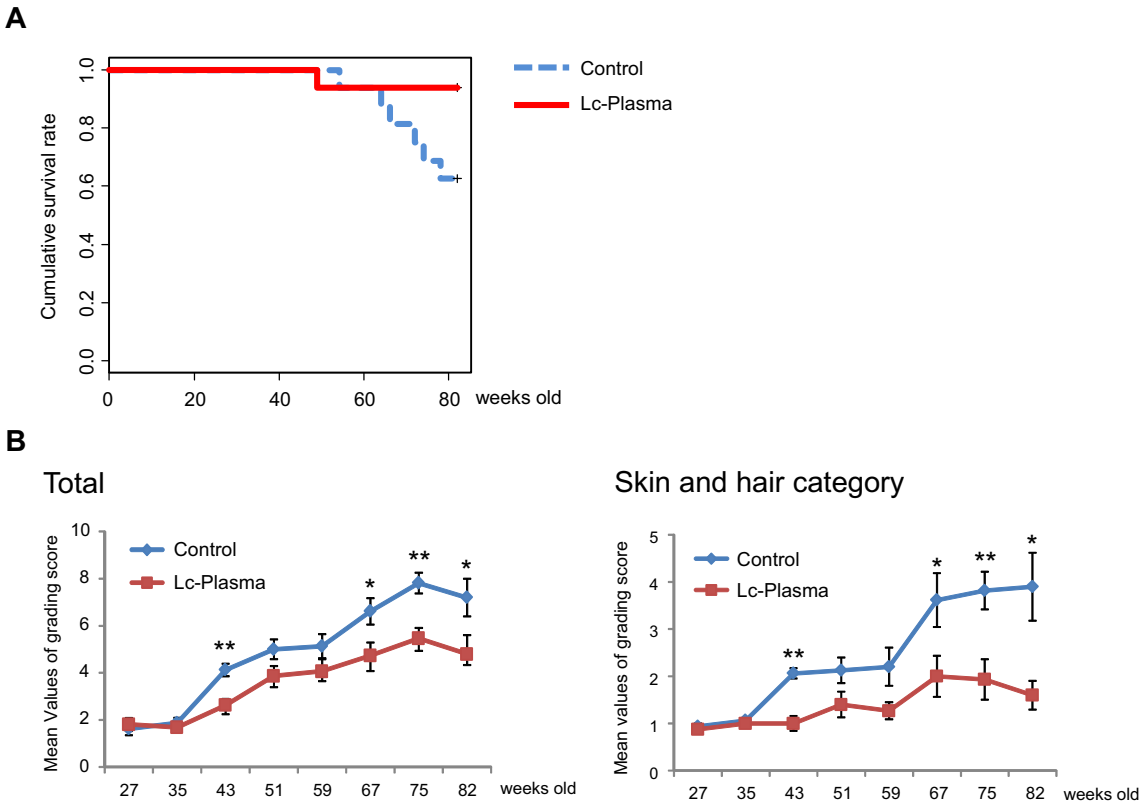

Fig. 1. Comparison of the cumulative survival rate and chronological changes in the degree of senescence.
The cumulative survival rate was evaluated at age 82 weeks (A). The control (dot-line) and Lc-Plasma (line) groups each consisted of 16 mice. $P < 0.05$ (Log-Rank test).
The degree of senescence 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