

論文の内容の要旨

応用生命化学 専攻
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論文題目 Studies on altered immune functions and their mechanisms during aging process
(加齢による免疫機能の変化及びそのメカニズムに関する研究)

Aging is sometimes accompanied by age-related diseases and it also causes alterations of organ systems, response to antigen, and cellular maintenance pathways, which result in impinging on the individual's quality of life. The ability to resist against pathogens, diseases or injury decreases with aging and some of the noticeable features are alterations in the development and maintenance of immune response and cellular function. These age-associated alterations are referred as immunosenescence, which means the state of changed immune function with aging. The aim of this study was to unveil the mechanisms of immunosenescence by examining the altered immune functions and responses with aging. I investigated the roles of aging on functional changes of immune response on cellular level and explored several substances that have an ability to modulate the age-associated altered immune functions.

1. Splenic stromal cells from aged mice produced high level of interleukin (IL)-6 during aging.

Proinflammatory cytokines, such as tumor necrosis factor (TNF)- α , IL-1, and IL-6 are significantly related to the age-associated diseases and disability, and these are important factors on inflammatory response in the elderly. IL-6 is detected in high levels in disease conditions including autoimmune disease, such as rheumatoid arthritis, atherosclerosis, and diabetes. It implies IL-6 might play roles that boost the risk and mortality in inflammation in the elderly. The principle objective in this chapter was to identify cell types that were affected by aging concerning secretion of IL-6 in the murine model. To answer these questions, I compared IL-6 production in splenocytes from both young and aged mice, isolated a number of cell types from spleen, and investigated IL-6 mRNA or protein expression. Stromal cells, nonhematopoietic cells giving structural support to lymphoid organs, have been considered as cells that do not significantly affect the immune response. However, recently the functions of stromal cells in

immunehomeostasis and immune responses have begun to emerge. It was newly found that IL-6 production of CD45⁺ stromal cells was greatly increased in aged mice. In addition, IL-6 mRNA was expressed highly in freshly isolated CD45⁺ stromal cells from aged mice, which alludes to the function of stromal cells in secreting IL-6 changes with aging. The distribution of subsets of these CD45⁺ stromal cells changed with aging. Blood endothelial cells (BECs) showed increased population in CD45⁺ stromal cells of aged mice. Altered distribution of CD45⁺ stromal cells, especially increased population of BECs might contribute to augment IL-6 production. The present results that increased IL-6 expression in splenic CD45⁺ stromal cells from aged mice suggest general etiological mechanisms of the chronic inflammatory condition found in the elderly, referred to inflamm-aging.

2. Mitochondrial DNA mutation augmented IL-6 expression.

In the nucleus and mitochondria, genome errors are accumulated in the elderly and these errors harm organelles. Here, the effect of mitochondrial damage on IL-6 production was investigated with aged D275A mice. D275A mice, which have two-base substitutions in the mitochondrial DNA polymerase gamma DNA, show impaired proofreading ability and increased spontaneous mutation rate in mitochondrial DNA, and these mice have a shorter lifespan than wild type (WT) mice. Spleen cells from D275A mice expressed higher levels of IL-6 mRNA compared to WT mice after lipopolysaccharide (LPS) injection. Since NF- κ B p65 mainly regulates IL-6 gene expression in LPS stimulated condition, I compared p65 protein levels by western blot after separation of splenic nuclear extracts from WT and D275A mice. However, NF- κ B from aged WT mice was highly activated compared to D275A mice and it implies that IL-6 might be induced in D275A mice in different pathway from WT mice. Mitochondria, involved in a wide range of cellular processes, including ATP generation, apoptosis, lipid metabolism, and interconversion of amino acids, are packed with various redox carriers and centers that can leak single electrons to oxygen and switch it to superoxide anion, one of the reactive oxygen species (ROS). Since TNF- α is known to induce ROS production, I investigated TNF- α expression level and ROS generation in D275A mice. The expression of TNF- α was two-times higher than WT mice after LPS injection and as expected, superoxide anion was highly produced in D275A compared to WT mice. These results imply that mitochondrial mutation might induce increased TNF- α expression upon LPS stimulation and results in further ROS generation in D275A mice and up-regulation of IL-6 mRNA expression levels. The present results have various implications for future studies on the function of ROS and mitochondria in inflammatory responses in the elderly.

3. Black garlic (BG) and *Cordyceps sinensis* extracts suppressed IL-6 production.

Garlic and *Cordyceps sinensis* have been widely used in traditional medicine, and it is believed that these dietary supplements have good effects on a number of disorders and infectious diseases. Garlic has numerous physiological and biological functions, such as reducing blood pressure and lowering down cholesterol levels. *C. sinensis*, a kind of fungus, has been used for treatment of asthma and inflammation, and affects several immune cells. Both BG and *C. sinensis* have been reported to influence immune responses by affecting production of cytokines. The main object in this chapter was to determine whether these substances have a reducing effect on IL-6 production in LPS stimulated splenocytes from aged mice. BG and *C. sinensis* suppressed IL-6 secretion but did not affect other cytokine productions such as IFN- γ or IL-2. IL-6 produced by stromal cells was also decreased by addition of BG but the effect of *C. sinensis* to suppress IL-6 production of stromal cells was not significant. These results imply the possibility that *C. sinensis* down-regulated LPS-induced IL-6 production by affecting immune cells such as macrophages or dendritic cells (DCs). On the other hand, BG acted on splenic stromal cells and as a result, IL-6 secretion of splenocytes was significantly reduced after LPS stimulation. The present results show that *C. sinensis* and BG have beneficial effects on treatment of inflammation. Suppression of IL-6 production in the elderly with these substances might be a good strategy to relieve or reduce symptoms triggered by inflammatory dysregulation such as rheumatoid arthritis, and various autoimmune diseases.

4. Attenuation of migration properties of CD4⁺ T cells from aged mice correlated with decreased chemokine receptor expression.

The influence of aging on CD4⁺ T cell migration ability toward chemokines was investigated with young and aged mice. The functional decline in migration ability toward CCL19 and decreased CCR7 expression level in antigen-stimulated CD4⁺ T cells from aged mice was newly found. It is known that retinoic acid induces the homing receptors for migration to the intestine. It was found that upon addition of retinoic acid (RA), CD4⁺ T cells from aged mice showed decreased CCR9 expression level, and the migration ability of CD4⁺ T cells from aged mice toward CCL25 was attenuated compared to young mice. I also observed that the expression of RALDH2 mRNA was decreased in mesenteric lymph node (MLN) DCs from aged mice compared to those from young

mice. In this chapter, attenuated migration abilities of CD4⁺ T cells from aged mice were observed, which correlated with decreased chemokine receptor expression. The reduced production and response to retinoic acid by aging may be one of the causes of such attenuated migration abilities in the intestinal immune system.

5. *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1 enhanced expression of RALDH.

Research on the beneficial effect of probiotics on immune system is performed for decades and probiotics have been considered as effective immune system modulators. Intake of milk products fermented with *L. bulgaricus* OLL1073R-1 has been shown to result in various biological effects, including immunomodulatory effects. The effect of *L. bulgaricus* OLL1073R-1 was investigated in aspect of intestine immune function in aged mice. Especially, the ability to improve immune functions by the action of RA was examined not only in the young but also in the aged with the murine model. The effect of *L. bulgaricus* OLL1073R-1 on RALDH mRNA expression levels was examined with MLN and Peyer's patch (PP) cells of young and aged mice and both young and aged mice showed upregulated RALDH2 level in MLN. *L. bulgaricus* OLL1073R-1 enhanced the RALDH expression level of MLN DCs, the result being consistent with MLN cells. The results show the possibility that by affecting RALDH expression level, *L. bulgaricus* OLL1073R-1 may modulate immune functions such as T cell migration and autoimmune diseases by inducing homing receptors or regulatory T cells. In this study, novel immunological functions of *L. bulgaricus* OLL1073R-1 to enhance RALDH expression was found, and this ability of *L. bulgaricus* OLL1073R-1 is likely to lead to the development of beneficial foods for the elderly to regulate immune responses.

6. Conclusion

The aim of present study was to clarify the age-associated altered immune functions and the mechanisms of those changes. In this study, several age-associated altered immunological functions were investigated. With aging, IL-6 secretion was up-regulated in stromal cells which maybe one of the reasons of occurrence of autoimmune diseases. Moreover, attenuation of age-associated T cell migration ability was observed. To elucidate the mechanisms of immunosenescence and search for beneficial foods for immunosenescence would be effective for improving the quality of life for the elderly.