Mini Review

Periodontal disease: Chronic low-grade inflammation accelerating aging

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Periodontal disease has been recognized as local infectious disease, which becomes major cause of tooth loss in the adults. However, it is now being re-recognized as low-grade inflammatory disease exhibiting negative impact on the host. It is believed that severer form of periodontal disease is often seen in diabetic and/or obese subjects. This may be associated with chronic immuno-activation due to hyperadipocytokinemia as well as hyperglycemia. Severe periodontal inflammation, in turn, acts to evoke insulin resistance and to accelerate atherosclerotic changes. Therefore, the disease may accelerate the fatigue of pancreatic β-cells as well as vascular inflammation. Overall, the disease may promotes the aging itself. Because of these unwanted negative effects of the disease, it is very important to understand the molecular mechanisms as to why such small, local inflammation due to oral infectious disease is intensified to the levels of influencing our systemic health. Here in this mini-review, we discuss about the negative effects of periodontal disease on acceleration of aging.

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Introduction

According to the estimation by 8020 Promotion Foundation in Japan, tooth loss due to the periodontal disease markedly increases after age 45 and about 41% of total tooth loss is due to the periodontal disease. As a result, about 20 to 25% of elder people aged 65 to 74 is edentulous. From these facts, it is obvious that periodontal disease is a disease of aged people. However, it is now being recognized that the disease itself may promote the aging as chronic low-grade inflammation.

We previously reported effective periodontal treatment mainly composed of antibiotic chemotherapy for diabetic subjects with severe periodontal disease results in the decline in circulating

tumor necrosis factor-α (TNF-α) and glycated hemoglobin value possibly via improved insulin sensitivity[1]. As periodontal disease is a chronic disease, it is possible that it will continuously lower insulin sensitivity if untreated properly, which ultimately accelerates the fatigue of pancreatic β-cells and may greatly affect longevity as suggested[2]. We also reported that serum antibody titer to the most well-known periodontal pathogen, *Porphyromonas gingivalis* is well-correlated with the elevation of serum c-reactive protein (CRP) level as measured by highly sensitive assay in non-obese Japanese type 2 diabetic subjects, indicating that periodontal infection up-regulates CRP value[3]. Also, periodontal treatment itself is reported to result in the decline in circulating CRP levels[4]. It is known that mild elevation of CRP well-predicts the future development of myocardial infarction[5]. Furthermore, anti-inflammatory therapy by statins targeted against apparently healthy subjects with mildly elevated CRP but with normal LDL-cholesterol level has recently been demonstrated to significantly reduce the risk of developing cardiovascular events, suggesting that low-grade inflammation plays an important role in developing such vascular disease, and approach lowering such inflammation is effective in reducing cardiovascular risk[6]. Importantly, elevation of CRP by periodontal disease is exactly in the range of such elevation. Furthermore, we and others suggested that periodontal infection is associated with early atherosclerotic changes[7,8] as well as micro- and macroalbuminurea[9,10]. Recently, it is reported that severe periodontal disease is strongly associated with the future cardio-renal death in type 2 diabetic subjects among Pima-Indians[11]. Because of these reports, it is very important to consider periodontal disease not only as the disease of the elder subjects but also as the disease which itself may promote the aging, and to understand the molecular basis as to why such local infection is intensified to the level of inflammation influencing our systemic health.

Amplification of inflammatory responses by hyperglycemia

To understand this important molecular mechanism, we first looked at the effects of hyperglycemia on macrophage function. We found that under hyperglycemic condition, THP-1 monocytic cells produces higher amounts of TNF-α and monocyte chemoattractant protein-1 (MCP-1). At the same time, c-Jun N-terminal kinase (JNK)-1 is hyperphosphorylated. As hyper-activation of JNK is known to associate with enhanced cytokine productivity, we used inhibition assay using JNK inhibitor. As expected, enhanced TNF-α and MCP-1 production was dose-dependently suppressed by using specific inhibitor for JNK in LPS-stimulated THP-1 cells cultured in high glucose containing medium[12]. It is well-known that hyperglycemia is associated with chronic activation of protein kinase C (PKC) in several cell types including monocytes[13]. Thus, we speculate that under hyperglycemic condition, both PKC and JNK are pre-activated in monocytes. And these cells respond to bacterial challenge in an exaggerated manner and produce higher amounts of inflammatory cytokines during infection.

Adipocyte-macrophage interaction

Recently, it has been reported that macrophages are integrated into adipose tissues, and interact with adipocytes, thereby producing higher amounts of adipocytokines[14]. Furthermore, both adipocytes and macrophages are reported to express toll-like receptor-4 (TLR4) and free fatty acids (FAA) appear to act as ligand for TLR4[15]. Although this is unique hypothesis when considering the pathogenesis of obesity-associated up-regulation of inflammatory changes, classical ligand for TLR4 is bacterial endotoxin, lipopolysaccharide (LPS). Additionally, it appears that infiltrated macrophages in adipose tissues are migrated via peripheral circulation. Therefore, we hypothesized that, in case of periodontal infection, activated macrophages via TLR4 by bacterial antigens may migrate into adipose tissues and interact with adipocytes to produce higher amounts of adipocytokines. Therefore, we established co-culture system between adipocytes and macrophages by using transwell system and stimulated these cells with bacterial LPS. In this condition, it was found that interleukin-6 (IL-6) and MCP-1 production was markedly enhanced in co-cultures stimulated with LPS as compared with each cell culture stimulated with LPS[16]. To see the role of macrophage-derived TNF-α in marked enhancement of IL-6 production, we performed neutralization assay by using neutralizing antibody for TNF-α. It was revealed that inhibiting TNF action partially (up to 50%) suppressed enhanced IL-6 production. From these results, we speculate that IL-6 enters the liver via portal vein to stimulate hepatocytes to produce CRP. MCP-1 acts to recruit more macrophages into adipose tissues. As TLR-4 ligand, LPS, markedly up-regulated cytokine production in co-cultures, we are interested to see differential gene expression in adipocytes co-cultured with macrophages in the presence or absence of LPS, and performed DNA microarray analyses. The results indicated that certainly, IL-6 and MCP-1 gene expression was markedly enhanced by LPS stimulation[17]. Besides IL-6 and MCP-1, we found marked up-regulation of many genes associated with inflammation and angiogenesis such as RANTES and CXC1/KC [17]. RANTES may promote the migration of T cells into adipose tissues, and CXC/

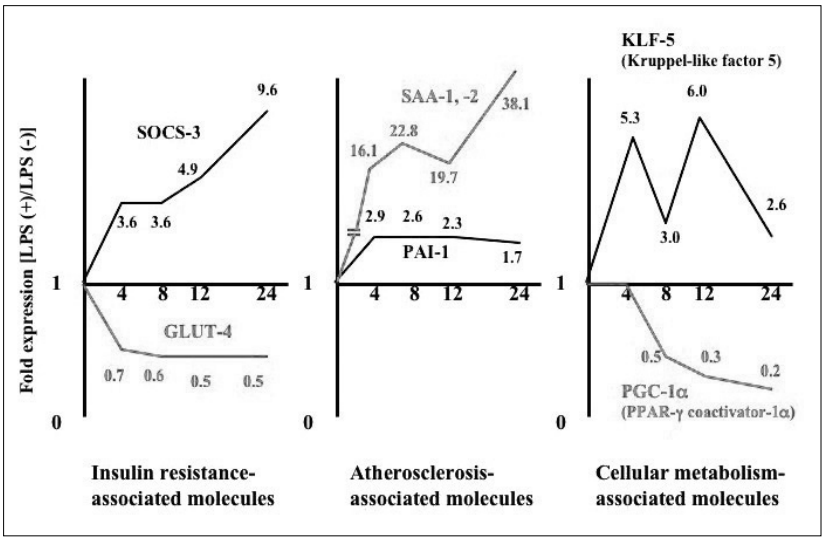

Fig.1 Relative expressions of SOCS-3, GLUT-4, SAA-1, -2, PAI-1, KLF-5, and PGC-1α mRNA in LPS-stimulated 3T3-L1 adipocytes co-cultured with RAW macrophages against those in un-stimulated adipocytes co-cultured with macro-phages[17]

Total RNA was isolated from adipocytes under each culture condition following 4, 8, 12 and 12h of LPS stimulation, and subjected to microarray analyses. Data are expressed as the fold expressions of genes in LPS-stimulated cells against those in un-stimulated cells.

KC may be associated with enhanced angiogenesis in the mature adipose tissues. As for the genes expression associated with insulin resistance, suppressor of cytokine signaling (SOCS) was up-regulated, while GLUT-4 expression was down-regulated. Serum amyloid A and plasminogen activator inhibitor-1 expression was up-regulated, suggesting that atherosclerotic changes are accelerated under such conditions. Interestingly, Kruppel-like transcription factor-5 expression is up-regulated, while PPAR-γ co-activator-1α (PGC-1α) gene expression was down-regulated, indicating that cell metabolism itself was suppressed (Fig.1)[18,19]. Overall, all of these differential gene expression acts to further promote inflammatory changes in the adipose tissues, to influence insulin sensitivity, to promote atherosclerotic changes and to suppress cell metabolism.

Periodontal disease-diabetes-aging axis: future directions

As indicated, local periodontal inflammation appears to be amplified to the level of influencing our systemic health, which might ultimately affect our longevity, especially in subjects with diabetes. However, to further confirm these important links, large epidemiological studies are necessary. One such study would be the one comparing lifespan between subjects with severe periodontal disease with elevated inflammatory markers such as CRP and the subjects without periodontal disease. Also, it would be nicer to compare physical as well as cognitive activity among subjects with or without periodontal disease. Additionally, further *in vitro* study investigating the amplification mechanism of inflammation in diabetic subjects other than adipocyte-macrophage interaction would be necessary, as there are many non-obese diabetic subjects in Japan as well as in Asian populations.

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