MINI REVIEW

The relationship between premature ageing and immune responses in the oral cavity of Down syndrome

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Summary Down syndrome (DS) is the most common chromosomal disorder resulting in various abnormalities such as mental retardation, immunodeficiency and physical abnormalities. Especially, abnormality of the immune function is important pathological features in this syndrome, and leads to increased susceptibility to viral or bacterial infections. Interestingly, several studies have showed that they have high susceptibility of severe periodontal disease, even though they have lower or equal prevalence of dental caries. Many studies have attempted to clarify this phenomenon but it remains unsolved. It is also well known that DS is a premature ageing syndrome. DS has been considered as a model of precocious, abnormal ageing of immune function in human. Age-related declines in immune function cause more susceptibility to infections in the elderly. In addition, it is well known that ageing is related with telomere shortening. Furthermore, DS has an accelerated telomere shortening. However, there are only a few reports that focus on the relations among ageing, telomere length and high susceptibility of oral infectious disease in individuals with DS. Therefore, we summarize our current knowledge on the relations between the oral disease and immunodeficiency in individuals with DS taking account of telomere shortening and ageing.

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1. Introduction

Down syndrome (DS), a premature ageing syndrome, is a chromosomal disorder caused by an error in cell division, resulting in the presence of an additional third chromosome 21 or ‘trisomy 21’ [1]. For this genetic disorder, DS results in a characteristic spectrum of developmental abnormalities affecting many organs and tissues [2]. It is known that DS results in mental retardation, short stature, abnormalities with cardiovascular, hematopoietic, musculoskeletal, nervous, and behaviour anomaly. Therefore, many studies have reported that individuals with DS have been prone to develop...
infectious, malignant, and autoimmune disease [3]. It has also been demonstrated that individuals with DS have many intraoral characteristics. According to Amano et al. [4], nine major abnormal dental characteristics in individuals with DS have been reported, including macroglossia, fissured tongue, underdeveloped maxilla, tongue thrusting, congenitally missing teeth, malocclusion, salivation, and microdontia [5,6]. As well as these features, it is well known that they have precipitation of a high calculus, severe periodontal disease, and a lower prevalence of dental caries. Periodontal disease and dental caries, which are the most common dental disease for human, are both a chronic infection by bacteria of normal oral flora. Namely, it is conceivable that individuals with DS have an imbalance in host response to bacteria. Several previous studies have suggested that genetic abnormalities in their host response, such as functional defects in polymorphonuclear leukocytes, monocytes, and lymphocytes, might be important contributing factors to the high prevalence of periodontal disease in individuals with DS [7–15]. Most have revealed that the prevalence of dental caries in individuals with DS was lower than in normal individuals, or individuals with other causes of mental retardation [16,17], even if there have been some studies that held an opposite view [18,19]. Thus, there is a diversity of views on oral status in individuals with DS. Moreover, the question why DS causes a high prevalence of periodontal disease and low prevalence of dental caries in individuals with DS is still unclear. Although many studies have attempted to clarify this phenomenon, there are few studies that focus on the relation among oral infection, immune response and acceleration of ageing in individuals with DS. Therefore, we summarize our current knowledge on the relations between the oral disease and immunodeficiency in individuals with DS taking account of telomere shortening and ageing.

2. Oral status

It has been demonstrated that individuals with DS have many intraoral features such as macroglossia, fissured tongue, underdeveloped maxilla, tongue thrusting, congenitally missing teeth, malocclusion, salivation, microdontia, high prevalence of severe periodontal disease, and low prevalence of dental caries. Above all, one of the most interesting is that individuals with DS have a high prevalence of severe periodontal disease, even though they have lower prevalence of dental caries. Dental caries and periodontal disease as well as many diseases of the mucous membranes, tongue, and salivary glands are infectious diseases. Lee et al. [20] reported that the difference in the prevalence of caries between individuals with DS and healthy controls was likely to depend on not the diet and oral hygiene habits, the occlusal feature of the teeth, and the interdental spaces, but on the immunoglobulin concentration level in the saliva. Further, Reuland-Bosma et al. [11] showed that under identical conditions with respect to food, hygiene and environment, a similar plaque development gave rise to a more extensive gingival inflammation in a child with DS than that in her sibling. They demonstrated that helper/inducer cells on the tissue level in a child with DS were stimulated earlier and during a longer period than those in her sibling. According to these findings, it is conceivable that there is an imbalance between inflammatory and anti-inflammatory networks in individuals with DS.

3. Salivary factors

It is well known that human saliva has been suggested to play an important role in protection against noxious compounds produced by microorganisms. In particular, secretory immunoglobulin A (sIgA) is major factor for the local host defence against dental caries and periodontal diseases. Several studies have demonstrated the relationship between IgA and periodontitis. Most studies have indicated that value of sIgA in individuals with severe gingival inflammation were lower than that in control [21]. In the generalized early-onset periodontitis, which is associated with the periodontitis of DS, a significantly lower concentration and secretion rate of total salivary IgA has been observed [22]. In other hand, Lee et al. [20] demonstrated that the individuals with DS had a significantly higher level of specific antibodies against Streptococcus mutans than the controls. Namely, it is suggested that children with DS have a lower prevalence of dental caries than normal children, which might be due to the higher amounts of S. mutans-specific IgA antibodies, not total IgA. Interestingly, Chaushu et al. [23] reported that whole saliva flow in old individuals with DS significantly reduced compared with young individuals with DS, while no statistically significant differences were found in the whole saliva flow rate of young control versus old control individuals. Although they examined the flow rate in parotid, there was no dramatic difference with age. They suggested that the secretory immunity in the oral cavity of individuals with DS was characterized by a severe immunodeficiency which was more severe in whole saliva than in parotid saliva, and was extremely exacerbated with age.

In general, the prevalence of infections derived from mucosa appears to increase with age. In individuals with DS, the prevalence and severity of mucosal gastrointestinal and respiratory infections increases with ageing [3,24–28]. These infections are associated with complete or transient deficiency of sIgA [3], although the levels of sIgA increase with age [29]. It is well known that individuals with DS have an acceleration of ageing. Thus, it is conceivable that an increase of sIgA in individuals with DS is caused by acceleration of ageing resulting from genetic abnormality. However, Percival et al. [30] suggest that the ability to form IgA antibody responses is not impaired with increased age, and that secretion rates and functional properties of antibodies may be as important as concentrations in protection against mucosal infective diseases. Although there are many reports that support immunodeficiency in saliva with increased age in individuals with DS, more sufficient evidence is required in future studies to clarify the role of sIgA in oral infectious disease.

4. Immune response

Although the reasons for an increased susceptibility to infections in individuals with DS are still not clear, it has been showed that individuals with DS have a defect in immune function resulting in an increased susceptibility to infections, a high risk of malignancies, and a high frequency of autoimmune phenomena [31–34]. Recently, several studies that have focused on abnormalities of the immune system in individuals with DS have been performed, demonstrating
selective cell-mediated immunodeficiency, defective neutrophil polymorphonuclear leukocyte chemotaxis, and impaired antibody response to specific pathogens, low T-cell lymphocyte counts, and immature subsets of T lymphocytes [17, 35–37]. Burgio et al. [38] performed the immunologic studies in 83 patients with DS in ages ranging from a few months to 30 years old. They indicated that both thymus-dependent and independent functions were impaired in patients with DS. They also indicated that serum immunoglobulin levels were normal in children with DS less than 5 years old, but a definite hyperglobulinemia of the IgG and IgA type was observed after 6 years of age. Furthermore, high levels of IgG1 and IgG3 were found, whereas a progressive decline of IgG2 and IgG4 with age was observed [39]. According to Kusters et al. [40], these phenomena are supported by a report that adults with DS have significantly higher percentages of interferon gamma-producing CD4+ and CD8+ cells and a higher Th1/Th2 ratio [41]. T helper lymphocyte type 1 cells (Th1) stimulate cytotoxic T lymphocyte response and IgG1 and IgG3 production, whereas T helper lymphocyte type 2 cells (Th2) stimulate antibody response by B lymphocyte cells and formation of IgG2 and IgG4. On the other hand, Cossarizza et al. [42] studied phenotype and proliferative ability of peripheral blood lymphocytes (PBLs) in individuals with DS. They indicated that a complex derangement of all major peripheral blood cell subsets, i.e., B cells, T cells, and natural killer (NK) cells, was present in a higher percentage of children with DS. A significant decrease of the absolute number of circulating lymphocytes, a marked and significant decrease of B lymphocyte absolute number and percentage, and dramatic modifications of the T-cell subsets were observed. The absolute number of CD4+ cells was significantly decreased, whereas CD8+ cells increased significantly in percentage but not in absolute number. Many of these alterations are similar to those characteristics of chromosomally normal subjects of advanced age [43]. Guazzarotti et al. [44] reported that a complex skewing of post-thymic lymphocyte maturation pathways was observed in individuals with DS: significant reduction of CD4+ and CD8+ naive T lymphocytes, significant increase of CD4+ and CD8+ central memory T lymphocytes and terminally differentiated lymphocytes. DS is a premature ageing syndrome, and thus it is conceivable that abnormal immuno response is in consequence of accelerated ageing. Immuno responsiveness undergoes a substantial decline with advancing age, particularly thymus-dependent immunity [45–48]. It is known that age-related declines in immune function cause susceptibility to infections resulting in increased morbidity and mortality in the elderly as well as in individuals with DS. In general, functions of T lymphocytes and B lymphocytes vary with ageing. Haynes and Maue [49] reported that declines in the function of CD4 T cells with ageing are thought to contribute to the decline in high affinity antibody production in the elderly. Age-related defect in CD4 T cell differentiation results in the generation of a group of T helper lymphocyte (Th) cells with significantly reduced B cell helper activity [50]. Therefore, it leads to reduce antibody production by B cell resulting in high susceptibility to infections. Tam and Walford [51] indicated that the cAMP/cGMP ratio for unstimulated T cells declined in normal aged and DS subjects. They indicated the existence of substantial age-related biochemical changes in peripheral T cells. And they suggested that an imbalance in resting cyclic nucleotide levels and their generating enzymes in T cells of normal aging and DS subjects might contribute to the immune dysfunction occurring both with aging and in DS. It has been thought that there were defects in B cells as well as in T cells during ageing. Frasca and Blomberg [52] indicated that elderly people have lower percentages of CD19+ total B cells and Kusters et al. [40] reported that a significant decrease of B lymphocytes (CD19+) was observed in fetuses with DS.

As stated above, several studies have reported various immunological theories and observations to explain the predisposition of individuals with DS to various infections. Most have focused on the abnormal role of the thymus in individuals with DS. Interestingly, Elsayed and Elsayed [53] focused on the precarious aging theory which increased apoptosis. Apoptosis plays a key role in tissue and organ development during embryo genesis as well as in adult tissues during cell turn-over. In adaptive immune response, after clearance of the antigen, most of effective T cells undergo apoptosis and only some survive to become long-lived memory T cell [54]. Elsayed et al. attempted to identify the effect of apoptosis on both types of cells of specific immune response (T and B lymphocytes) in children with DS using Annexin V staining phosphatidylserine, which can detect early apoptosis. In their work, both relative and absolute number of early apoptotic T lymphocytes was significantly lower in children with DS, while the absolute number of T lymphocytes was insignificantly different. They concluded that increased early apoptotic cells in children with DS might emphasize the fact that the function of cell, and not their number, was the main mechanism responsible for the impairment of the immune system in children with DS.

There are two interesting studies about oral infections in individuals with DS. Carlstedt et al. [55] studied the oral carriage of Candida albicans (C. albicans) in 55 children and adults with DS, aged between 7 months and 20 years 6 months. The number of subjects with colonized C. albicans in oral cavity was significantly higher in the DS group than in the control group, and the frequency of colonization with C. albicans was positively correlated to age. They suggested that abnormalities of the immune response in individuals with DS might be contributed to the increased oral carriage of C. albicans. According to Ribeiro et al. [56], who studied to characterize the biological aspects of oral strains of C. albicans in children with DS, all oral C. albicans isolated were sensitive to the drug used and this characteristic was also observed in C. albicans obtained from patients with other immunodeficiencies such as HIV, cancer, and diabetes. As ageing leads to a decline in the function of the immune system, the prevalence of fungal infections, such as C. albicans, increase dramatically among the aged population. Thus it is conceivable that it is possible that the susceptibility to the mucosal infections is caused not only by the innate abnormal immune response, but also by acceleration of ageing in individuals with DS.

Taken together, it is more likely that an abnormal immune response in individuals with DS is congenital and is intrinsically deficient from the very beginning. However, several previous studies have indicated that immune response become deranged during ageing and it is conceivable that immunodeficiency in individuals with DS is caused as a consequence of precocious ageing in DS.
5. Telomeres factors

Telomeres, chromosome ends consisting of highly conserved TTAGGG repeats, become shorter with every cell division [57]. The main function of telomeres is to cap the chromosome ends to distinguish the chromosome ends from DNA breaks within the genome [58]. Telomere shortening accompanying cell division was shown to occur both in vitro and in vivo with age reflecting the cumulative effect of cell division [59]. This phenomenon was demonstrated in human fibroblasts [60], hematopoietic stem cells [61], leukocytes [62], keratinocytes [63], epithelial [64], and endothelial cells [65]. To produce telomere elongation, an enzyme called telomerase exists. Telomerase is a cellular reverse transcriptase and consists of two essential components: (1) the telomerase RNA and (2) telomerase reverse transcriptase (TERT) [66]. hTER (human TER) provides the template for repeat synthesis and is constitutively expressed in human cells, and most somatic cells repress hTERT (human TERT) expression at the transcriptional level [67]. Most somatic cells in adult organisms do not express telomerase and this enzyme is active only in germ cells, during embryogenesis, in adult stem cells and in activated immune cells, suggesting that its activity is tightly regulated during development and differentiation [68]. Most normal somatic cells exhibit capacity for only a finite number of divisions in vitro before reaching replicative senescence, a well-known phenomenon called the Hayflick limit. Once telomeres are critically shortened, cells cease to divide and become senescent or undergo apoptosis. In other words, telomeres play an essential role in determining the replicative lifespan of cells.

5.1. Telomeres and immunosenescence

It has been reported that increased telomere shortening has been associated with a variety of conditions that include replicative cellular senescence, apoptosis [57,69], osteoporosis [70], and Down syndrome [71]. Moreover, Jiang et al. [72] reported that individuals with short telomeres had an 8.54-fold higher mortality rate from infectious disease compared to those with relatively long telomeres. According to their report, telomere shortening was observed in T lymphocytes and progenitor/stem cell in individuals with DS [71,73]. In addition, Vaziri et al. [74] showed a significantly higher rate of telomere loss in peripheral blood lymphocytes (PBLs) in individuals with DS compared to those in control and was accelerated with aging. They also indicated that telomere length of lymphocytes from centenarians and older individuals with DS were similar to those of senescent lymphocytes in culture, which suggests that replicative senescence could partially account for ageing of the immune system in individuals with DS and in the elderly. Immunosenescence exert an influence on many components of the immune response, and it has been indicated that accelerated telomere shortening of lymphocytes was associated with the premature immunosenescence in individuals with DS [74,75].

5.2. Telomeres and fibroblasts

Telomere length has been determined in a variety of tissues and it was observed that telomere length varies considerably from tissue to tissue. Cells in different tissues differ enormously in their turnover rate and it is known that telomeres in human fibroblasts shorten 50–100 basepairs with each cell division [57]. The replicative capacity of the cell is predicted by initial telomere length as well as most somatic cells [57,60].

We established gingival fibroblasts from individuals with DS (DGF) which were immortalized by transduction with hTERT gene (hTERT-DGF) using modified protocol of immortalized periodontal ligament cell [76]. DGF and hTERT-DGF were stimulated with lipopolysaccharide (LPS) derived from P. gingivalis. We found that the production of interleukin-6 in hTERT-DGF was significantly lower than that in DGF in the presence of LPS (unpublished data). It is conceivable that the effect of LPS was suppressed in DGF produced telomere elongation because of hTERT transduction. However, we should hypothesize with circumspection. There are two noteworthy studies. Kimura et al. [77] explored cellular proliferation and senescence on cultured skin fibroblasts from individuals with Down syndrome and healthy individuals. They reported that there were no significant differences of the cumulative number of population doubling until replicative senescence and β-galactosidase staining, which was known as a biomarker for cellular senescence, between DS cells and control cells. Takahashi et al. [78] reported that there were no significant differences in the mean telomere length of PBL and human gingival fibroblasts between the patients with aggressive periodontitis and the controls. They suggested that aggressive periodontal disease was not a disease of generalized accelerated aging and that accelerated telomere loss was not a useful marker of premature immunosenescence of patients with aggressive periodontal disease. Therefore, this is negative toward our hypothesis which accelerated telomere shortening causes severe periodontal disease in individuals with DS. However, they did not study telomere length in DGF. And, according to the previous papers, it is conceivable that telomere length have an effect on immune response and other intracellular functions. Furthermore, telomere length is sensitive to genetic factors and environmental factors like chronic inflammation and exposure to infectious agents. In addition, as the investigation related with ageing and periodontal disease is the beginning, there are few reports. Therefore, further study will be needed.

5.3. Telomeres and oxidative stress

Presently, some studies indicate that chronic oxidative stress plays a major role in chronic inflammatory disease. Kawanishi and Oikawa [79] investigated whether oxidative stress, which contributed to aging, accelerated telomere shortening in human cultured cells. Telomeres were demonstrated to be highly sensitive to damage by oxidative stress due to their high content of guanines. In addition, Passos [80] reported that senescent cells had higher reactive oxygen species (ROS) levels and indicated mitochondrial production of ROS as one of the causes of replicative senescence. They suggested that mitochondrial ROS was a major determinant of telomere-dependent senescence at the single-cell level that is responsible for cell-to-cell variation in replicative lifespan. In individuals with DS, some of the manifestations of accelerated aging have been attributed to an imbalance between the first (dismutation) and second (conversion of hydrogen...
peroxide to water by Gpx and catalase) step in pivotal enzymatic pathway defending against ROS [77,81]. There is one report that studied the influence of ROS on periodontitis of individuals with DS. It suggested that this might due to the enzymatic ability of over-expressed CuZn-superoxide dismutase in individuals with DS to catalyze the formation of H$_2$O$_2$ from O$_2^*$, thereby increasing the availability of substrate H$_2$O$_2$ for the iron-dependent generation of HO* via the Fenton reaction, suggesting that HO* generated from DS-GF may be involved in progressive periodontitis of DS [82].

6. Conclusion

Many investigators have attempted to elucidate the imbalance in oral phenomena in individuals with DS. The question is why they have high prevalence of periodontal disease and lower or equal prevalence of dental caries in comparison to normal individuals. However, the opinion has been divided on this question. In this review, we have attempted to hypothesize that accelerated ageing caused by telomere shortening leads to immunodeficiency resulting in the imbalance in oral phenomena in individuals with DS (Fig. 1). Unfortunately, there are some negative views about the relations among telomere shortening, ageing, and inflammation. However, there are many positive reports to elucidate that telomere shortening causes immune deficiency in the elderly and in individuals with DS (Tables 1 and 2). Therefore, we cannot deny our hypothesis, and we believe that this hypothesis will be supported by sufficient evidence in the near future. In addition, Sommer et al. [83] analyzed global changes of gene expression in lymphocytes from children with DS by means of the serial analysis of gene expression and indicated that trisomy 21 induced a modest dysregulation of disomic genes that might be related to the immunological perturbations seen in DS. Therefore, further studies are needed to

![Diagram](Figure 1) Etiological hypothesis in abnormal immune response of Down syndrome. Abnormal immune host response, premature ageing and environmental stimulation lead to a vicious circle, which finally occur to severe periodontal disease.

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elucidate our hypothesis and should be discovered by genetic and environmental background.

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