

Chapter 2

The Role of Protein Oxidative Modification in Periodontal Diseases

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2.1 Introduction

As is explained elsewhere in this book, almost all biological macromolecules including DNAs, RNAs, lipids, carbohydrates, and proteins are oxidated. In most cases, oxidation means damage, but recent discoveries have shown that oxidation of biomolecules is playing important roles in fine-tuning of our body as well, for example, regulations of transcription factors and signal transductions. At the same time, it has also been becoming clear that imbalance of such fine-tuning can lead to complicated diseases.

In this regard, involvement of oxidative stress-induced post-translational modification (OPTM) in various disorders is drawing the interest in research. Different from other post-translational protein modifications such as glycosylations or phosphorylations, which are programmed to occur as a part of protein maturation processes in our body, OPTM implies alterations or degradation of matured proteins. OPTM of proteins is enhanced in aging and stressed cells and arises under physiological conditions [1, 2].

Target readers of this review article are those who are interested in periodontal disease (researchers in the field of periodontology and dental students) and would like to enter the field of oxidation research. For advanced readers, other excellent review articles [1, 3, 4] on oxidation of other biomolecules or effects of reactive oxidative species (ROS) are available and are highly recommended. The goal of this review article is to provide basic information and concept of oxidative protein modification to the newcomers of this field.

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2.2 Basic Knowledge About Protein Oxidation

2.2.1 Definition of the Oxidation

In our body, protein oxidation can occur both enzymatically or non-enzymatically. The former is specific catalytic function of many enzymes in substrate-specific manner and usually is a part of physiological functions of our body. The latter is rather nonspecific and can cause unwanted effects to our body leading to various disorders. In this review, therefore, we set aside the former and focus on the latter.

Protein oxidation can be defined as the covalent modification of protein induced either directly by ROS or indirectly by reaction with secondary by-products of “oxidative stress” [5]. ROS can be defined as “molecules and ions of oxygen that have an unpaired electron, thus rendering them extremely reactive.” Oxidative stress is defined as “a disturbance in the pro-oxidant–antioxidant balance in favor of the former, leading to potential damage” [3]. In other words, oxidative stress occurs when the balance of formation of oxidants exceeds the ability of antioxidant systems to remove ROS, which occurs when inflammatory phagocytes (e.g., neutrophils and macrophages) are activated to undergo an oxidative burst by exposure to a foreign agent or when pro-oxidant xenobiotics are introduced into the body. Under these conditions, biomolecules become subjected to attack by excess ROS and significant molecular and physiological damage can occur.

Free radicals have been defined as “any species capable of independent existence that contain one or more unpaired electrons” [6]. The term “free radical” and “ROS” is almost the same, but “ROS” has become more popular because it encompasses other reactive species which are not true radicals but are nevertheless capable of radical formation in the intra- and extracellular environments. The primary free radical in most oxygenated biological systems is the superoxide radical ($O_2^{\cdot-}$), which is in equilibrium with its protonated form, the hydroperoxyl radical ($HO_2^{\cdot}$). Although $O_2^{\cdot-}$ is relatively unreactive in comparison with many other radicals, biological systems can convert it into other more reactive species, such as peroxy ($ROO^{\cdot}$), alkoxy ($RO^{\cdot}$), and hydroxyl ($HO^{\cdot}$) radicals.

2.2.2 S-Nitrosylation

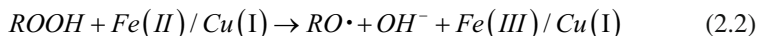
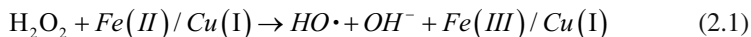
According to the above-mentioned definition, molecular oxygen (O_2) is not always necessary for oxidation, as long as the transfer of electrons occurs. Thus many different biochemical reactions, which include nitrosylation, peroxidization, are known to oxidate target molecules. Among them, S-nitrosylation is famous for its biological significance.

S-nitrosylation is the covalent attachment of a nitrogen monoxide group to the thiol side chain of cysteine. This has emerged as an important mechanism for dynamic, post-translational regulation of most or all main classes of protein.

S-nitrosylation thereby conveys a large part of the ubiquitous influence of nitric oxide (NO) on cellular signal transduction and provides a mechanism for redox-based physiological regulation [7].

2.2.3 Metal-Catalyzed Oxidation

Theoretically, any one of the pro-oxidant systems is able to oxidate proteins; however, there is reason to believe that under normal conditions the metal-catalyzed oxidation systems are the major source of oxidation. This follows from the fact that hydrogen peroxide and alkylperoxides are the most common end products of most ROS-generating systems. These peroxides by themselves are relatively unreactive compounds. However, in the presence of the transition metals, Fe(II) or Cu(I), they are converted to the highly reactive hydroxyl radical [reaction (2.1)] or alkoxy radical [reaction (2.2)] which are capable of reacting with almost any organic substance.



Indeed, virtually all kinds of amino acid residues of proteins are potential targets for oxidation by HO· generated by ionizing radiation or by high concentrations of H₂O₂ and Fe(II) [8, 9]. However, at the low concentrations of iron or copper ions and H₂O₂ present under most physiological conditions, protein damage is likely limited to the modification of amino acid residues at metal-binding sites on the protein, which effectively concentrate the ions. This consideration gave rise to the proposition that the oxidation of proteins under physiological conditions is a site-specific process in which the binding of Fe(II) or Cu(I) to metal-binding sites on the protein is followed by reaction with peroxides to generate reactive species (OH·, RO·, per-ferryl radical) that will react preferentially with amino acid residues at the metal-binding site [10–13]. Thus, the metal-binding site supports a biologically important “caged reaction.” In the case of many enzymes, especially with those requiring a metal ion for activity, this will lead to loss of catalytic function [14].

2.2.4 Anti-oxidation System

Under normal circumstances, there is a well-organized balance between formation and neutralization of ROS so that there is minimal modification of biomolecules. Such balance is achieved by antioxidant systems which exist in our cells and tissues to scavenge or otherwise eliminate them. These include antioxidant enzymes such as catalase, superoxide dismutase, peroxiredoxins, and glutathione peroxidase as well as low-molecular-weight compounds such as vitamins C and E and reduced glutathione (GSH) [5].

2.2.5 *Source of Pro-oxidants*

Nonenzymatic oxidation of protein is caused by oxidative radicals and ROS which can be provided from exogenous and endogenous sources. Exogenous sources include heat, trauma, ultrasound, ultraviolet light, ozone, smoking, exhaust fumes, radiation, infection, excessive exercise, and therapeutic drugs [15–17]. Endogenous sources are primarily:

- (1) By-products of metabolic pathways—electron leakage from mitochondrial electron transport systems forming superoxide;
- (2) Functional generation by host defense cells (phagocytes) and cells of the connective tissues (osteoclasts and fibroblasts).

Cell metabolism involves the consumption of oxygen and its utilization via glycolysis to form pyruvate within the mitochondria. The amino acid cycle follows and ATP is generated. However, electrons leak from their transporters at a constant rate, reducing oxygen to the superoxide anion. The incomplete reduction of oxygen is estimated at 1–3 % of consumed oxygen [18, 19] and at a rate that exceeds the mitochondrial antioxidant scavenger's ability to remove superoxide. Superoxide dismutase 2 functions to remove the superoxide radicals that form. Nevertheless, mitochondrial DNA damage by ROS and reactive nitrogen species (RNS) still occurs and is a process believed to be important in certain chronic diseases and in aging [20].

Functional production of superoxide involves activation of the hexose-monophosphate (or NADPH-oxidase) shunt, which shunts glucose-6-phosphate from the glycolysis pathway and utilizes molecular oxygen and NADPH to form the superoxide radical anion (O_2^-). This process comprises the so-called respiratory burst within neutrophilic polymorphonuclear leukocytes (neutrophils) and is stimulated by a variety of mitogens/antigens/cytokines and other mediators such as granulocyte-macrophage colony-stimulating factor. Respiratory burst is known as an important killing mechanism in cells after phagocytosis of bacteria. Important activators are opsonized particles which activate Fc γ receptors (Fc γ R), bacterial DNA which can activate Toll-like receptors (e.g. TLR-4, TLR-9) [21], small peptides from bacteria, such as fMetLeuPhe, and protein kinase C agonists, such as phorbol myristate acetate. The NADPH-oxidase has a complex structure including cytosolic sub-units (e.g. p47phox, p40phox, p67phox), and sub-units which are bound within the lipid membrane (e.g. gp91phox, p22phox). The proximal pathways that link the cell surface receptors to the oxidase differ in temporal behavior and biochemical components, but the downstream pathways seem to converge at the cytosolic activation points of the NADPH-oxidase [22]. Therefore bacterial infection enhances production of superoxide mainly from infiltrated neutrophils leading to generation of oxidated proteins.

2.2.6 *Biochemistry of Protein Oxidation*

Oxidative modification on proteins can occur both in the backbone and the side chain of a protein. Oxidative modification in the backbone usually results in

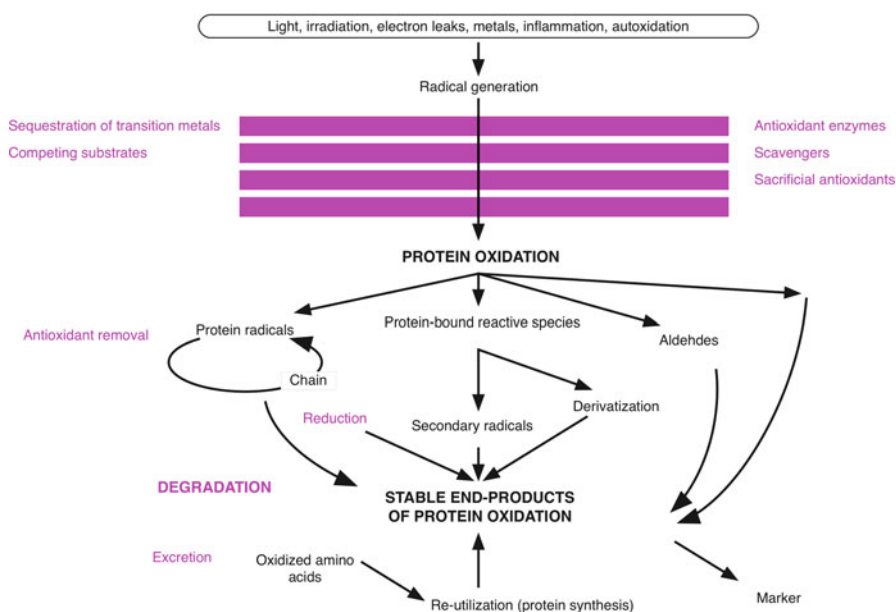


Fig. 2.1 Postulated mechanisms of protein modification in vivo. This figure is adapted from Dean et al. and shows postulated mechanisms of oxidative protein modifications in vivo. Processes depicted in color are those likely to result in the amelioration of protein damage in vivo

backbone fragmentation through primarily hydrogen atom abstraction at alpha carbon, while that in the side chain usually results in the formation of altered side-chains.

A variety of further consequences can occur after the initial oxidation of proteins. Usually the initial oxidation of protein caused by ROS leads to a formation of new reactive species (peroxides, DOPA) or further radicals which can oxidate sensitive biomolecules nearby. This reaction is repeated as a chain reaction until electrons of those ROS or radicals are absorbed by antioxidants. This process is often called propagation of oxidative effect.

The alteration of side chain of protein molecules can cause unfolding or conformational change. If this happens in multiple proteins simultaneously, dimerization or aggregation can also occur. Because structural conformation is critical for protein activities, those changes can affect in variety of functions of proteins. If this happens in enzymes or transcription factors, the functions of these molecules can be lost. This may affect numerous different cell functions including gene expressions, cell signaling, or induction of apoptosis and necrosis. Structural conformation is also important when proteins are recognized by protein processing systems in our body as well. Oxidative modifications of proteins can lead to alterations in cellular handling/turnover. This may further cause accumulation of abnormal proteins in cells which can be toxic [23, 24]. Above-mentioned consequences are summarized in a famous scheme (Fig. 2.1) created by Dean et al. [4].

2.2.7 *Biological Characteristics of Protein Oxidation*

For the most part, oxidatively modified proteins are not repaired and must be removed by proteolytic degradation, and a decrease in the efficiency of proteolysis will cause an increase in the cellular content of oxidatively modified proteins. The level of these modified molecules can be quantitated by measurement of the protein carbonyl content, which has been shown to increase in a variety of diseases and processes, most notably during aging. Accumulation of modified proteins disrupts cellular function either by loss of catalytic and structural integrity or by interruption of regulatory pathway.

2.2.8 *Investigation*

It is not easy to clarify the entire process in which protein modification affects. It is mainly because that protein oxidation is not a simple reaction. As mentioned above, oxidation often initiates multi-step chain reactions. Furthermore, intermediates in these reactions are highly unstable and have very short lives. Until recently, only possible way to detect the protein oxidation was measurements of protein carbonyls. Recently, increasing numbers of research tools have been introduced as exemplified by different monoclonal antibodies specific to oxidated motif such as malondialdehyde-oxidated-LDL, 4-hydroxy-2-nonenal (HNE), and hexanoyl-lysine (HEL). More and more developments of those useful tools are demanded.

2.2.9 *The Involvement of OPTM in Periodontal Disease*

One of the authors of this chapter has been exploring the involvement of OPTM in several different disorders. Several representative publications revealed that nitric oxide regulates exocytosis by *S*-nitrosylation or oxidation of Thiol-group on *N*-ethylmaleimide-sensitive factor (NSF) [25–29]. In another publication, it was demonstrated that innate immune response is controlled by the *S*-nitrosylation of MyD88 which is a key molecule of Toll-like receptor signal [30, 31]. Based on these experiences, it seems reasonable to think that OPTM is associated in the pathogenesis of PD as well as other chronic inflammatory diseases. Although numbers of papers have implied the involvement of oxidative stress in the pathogenesis of PD, those are mostly indirect and circumstantial.

In this review article, authors will focus on a new possible mechanism connecting OPTM and chronic inflammatory disease, which is breakdown of immunological tolerance. This mechanism is recently proposed [32, 33] and is implied to be involved in many chronic inflammatory diseases including atherosclerosis and autoimmune diseases. Authors would like to provide recent findings concerning this

mechanism and discuss about the possible involvement in the pathogenesis of both inflammatory bowel disease (IBD) and periodontal disease (PD).

2.3 The Role of Protein Oxidative Modification in Periodontal Diseases

2.3.1 Definition of the Target in This Discussion

As previously discussed, involvement of post-translational protein modification in various diseases has been proposed in recent years [1, 2]. In this chapter, we would like to discuss the possibility of its involvement in periodontal disease (PD). Before starting, we would like to define the target of our discussion. The term “periodontal disease” may involve a broad range of pathogeneses that arise in the area of periodontal tissue. In this review, we would like to use the term “periodontal disease” only to describe destructive chronic inflammation related to dental plaque, which consists of oral commensal bacteria and their biofilm. This category may include “chronic periodontitis”, “aggressive periodontitis”, and “periodontitis as a manifestation of systemic diseases” as they were identified in the most recent classification of periodontal disease by American Association of Periodontology [34].

2.3.2 The Changing Concept of the Pathogenicity of Periodontal Disease

In earlier days, when periodontal research was carried out mostly from the viewpoint of microbiology, periodontal disease was considered as an infection of specific causative bacteria and tissue destruction in this disease was thought to be caused by direct or indirect effects of specific pathogenic factors of those bacteria. Through the decades, however, this concept is changing. Nowadays, an increasing number of publications are suggesting that the direct effectors of the periodontal tissue destructions are components of the host immune system [35–41]. Although the old concept is still supported by many researchers, periodontopathic bacteria are now considered as a part of oral commensal bacteria rather than transient intruders.

All of the alimentary tract including oral cavity and colon lumen are covered by a heavy load of endogenous bacteria and are exposed to the flow of food. At the same time, mucosal surface of alimentary tract can be an entrance to foreign pathogenic bacteria. Therefore, it is currently accepted that antigen-specific immunological tolerance to those endogenous bacteria and food antigens exists so that systemic adaptive immune system would not overreact against those antigens, while at the same time maintaining reactivity to pathogenic bacteria [42].

It is still controversial if this “dogma” can be applied to oral commensal bacteria. For example, some researchers propose that immunological tolerance exists to oral commensal bacteria and breakdown of this tolerance may cause periodontal bone loss via RANKL-dependent manner [43]. Other researchers insist that *Porphyromonas gingivalis* (*P. gingivalis*), which is a representative periodontopathic oral bacteria, is not commensal bacteria, even though *P. gingivalis* can be isolated from healthy individuals. They explain that *P. gingivalis* is an opportunistic pathogen [44] rather than peaceful commensal bacteria. Thus, it may be fair to say that the recent concept regards periodontal disease as “chronic inflammatory disorder in which complex interaction of oral commensal bacteria and host immune system are involved.”

2.3.3 IBD: Another Complicated Disorder in the Alimentary Tract

There is another complicated disorder in which aberrant host immune responses against endogenous bacteria are involved in the alimentary tract. This is known as IBD. IBD is a chronic inflammatory condition of the intestine, which is characterized by rectal bleeding, severe diarrhea, and weight loss. The two major types of IBD are ulcerative colitis (UC) and Crohn disease (CD). IBD used to be found mainly in Western countries, namely Europe and the USA, but the numbers of cases are increasing in other areas of the world. Accordingly, reliable etiological data from those recently emerging world regions has not yet been available. What we do know is that currently up to 1.4 million Americans have IBD, with ~30,000 new cases being diagnosed each year. At present, there is no cure for IBD. Over the long term, up to 75 % of patients with CD and 25–33 % of those with UC will require surgery. Although the clinical symptoms are similar, the pathological characteristics of these two diseases are quite different. UC is characterized by confluent inflammation of the colonic mucosa, extending to a variable extent from the rectum to the proximal colon. CD, on the other hand, is characterized by discontinuous transmural inflammation, involving any portion of the gastrointestinal tract, but most commonly the terminal ileum [45]. Even with these different characteristics, these two diseases are considered to share the similar mechanism of pathogenesis.

In 1995, Duchmann et al. [46] introduced the idea that immunological tolerance exists toward intestinal commensal bacteria and this tolerance was broken in active IBD. Before this paper, the relationship of commensal bacteria to host immune system was unknown or was thought to be ignored by the immune system: because alimentary tract is still outside of the body, i.e. beyond the area to be protected by the immune system. This paper evoked lively arguments concerning the pathogenesis of IBD, where at times their theory was vigorously scrutinized, but the principle concept of immunological tolerance exist toward intestinal commensal bacteria remained unaffected. The current general agreement is that IBD is the result of the interplay of at least four factors: genetic predisposition, an altered immune response, the microbial flora of the gut, and environmental factors that may act as a trigger of the disease manifestations [38].

For the genetic predisposition, specifically, large-scale genetic investigations have revealed that mutation in ATG16L1 (autophagy-related 16-like 1) gene has strong relation to the pathogenesis of CD [47, 48].

2.3.4 IBD and PD: Are These Share Similarities?

In 2001, Brandtzaeg pointed out the similarity of periodontal disease to IBD. In his review article [49], he described “both inflammatory disorders (IBD and PD) can apparently be explained by endogenous infection inducing hypersensitivity against commensal bacteria.” His suggestion was, however, not well accepted in the dental research field. Investigations in line with this concept fell out of interest until 2010, when similarity of IBD and PD was finally revisited by Indriolo et al. [38].

One of the reasons why most researchers in periodontology field do not consider these two diseases to be similar may be due to the difference in their immunopathological characteristics. The periodontal disease lesion is characterized by the dominance of plasma cells and lymphocytes in the inflammatory infiltrate [50–57], while the majority of the infiltrates in IBD lesion are T cells. It should be noted that more detailed characteristics of the lesions of UC and CD are quite different as well. UC lesion is characterized by an atypical Th2 response, with elevated production of IL-13, while in CD predominates a Th1 type of immune response, dominated by overproduction of IFN-gamma. Even so, researchers in the field of gastroenterology still recognize them as the same pathogenesis. Therefore, it may be that periodontal researchers are simply overreacting to the pathological difference between IBD and PD. Perhaps periodontal researchers should set aside these differences and concentrate their effort on learning from the field of IBD research.

From the clinical point of view, both IBD and PD show similar natural history. In PD, the destruction of periodontal tissue does not occur at a constant rate: instead, there are cycles in which rapid tissue destruction occurs followed by slowing or even remission of the process. Socransky et al. [58] described this nature of PD and proposed a “random burst theory,” which has been well accepted until now.

On the other hand, IBD is a refractory disease and frequent relapses occur after certain period of remissions [38]. It should be of note that the progresses of both diseases are not continuous. This may imply that the manifestations of both diseases arise from imbalance of delicate homeostasis. The homeostasis of immunological tolerance to endogenous flora may be disrupted at the onset of the diseases and can be returned to its balanced form when the cause disappears or at least becomes weak.

The above-mentioned “random burst theory” also comprises the site specificity. Even within a dentition in a patient, aggressive tissue destruction can occur in one site (i.e. periodontal pocket) while the adjacent site shows no sign of destruction. Similarly, CD is known to form discontinued lesions along the gastrointestinal tract in terminal ileum. This may mean that certain local factors are involved in the initiation or progress of both diseases, but so far, there are no good explanations why such site specificities exist in both diseases. Further research is necessary in order to elucidate the natural history of these two diseases.

As described so far, the similarity of IBD and PD is not well recognized in periodontal research filed. Indriolo et al. stated in their review paper, “To our knowledge, this paper is the first review evaluating the possible common pathogenic pathways between PD and IBD.” The similarity of IBD and PD, however, seems more reasonable when the involvement of protein oxidation is taken into consideration.

2.3.5 Post-translational Protein Oxidization in IBD

As stated earlier, protein oxidation can be associated with aging and many chronic inflammatory diseases including atherosclerosis, rheumatoid arthritis, diabetes, and neurodegenerative diseases [59]. As new findings are accumulated year by year, several papers recently proposed that epitopes created through OPTM can induce a breakdown of immunological tolerance toward various antigens as well [32, 33, 60]. The underlying mechanism of this induction is molecular mimicry. Lipid and protein epitopes generated by lipid peroxidation (adducts) show cross-reactivities against DNA or epitopes expressed on cells undergoing apoptosis. Under normal conditions, antibodies against DNAs or apoptotic cells should not exist because they are self-reacting antibodies (Fig. 2.2). Oxidative modifications of biomolecules, however, may be able to generate new epitopes which have molecular similarity and may induce new antibodies which recognize self-antigens. These revelations expanded the possibility of OPTM involvement to chronic inflammatory diseases.

Some groups of researchers are also speculating the relationship between OPTM and IBD pathogenesis, but it has not been clearly demonstrated yet [45, 61].

Recent advancement in genetic research has provided supporting findings concerning OPTM involvement in IBD pathogenesis (Fig. 2.3). Etiological studies had implicated ATG16L1 protein to Crohn disease [48], thus suggesting that dysregulation of autophagy is one of the mechanisms of the pathogenesis of IBD. In 2008, Saito et al. clarified the mechanism of ATG16L1 in the pathogenesis of endotoxin-induced inflammatory diseases in mice. After that, involvement of more and more numbers of autophagy related genes was found in IBD patients.

It is currently considered that OPTM adducts are removed from our body by autophagy. If the function of autophagy is impaired in IBD due to other predisposed factors, it is plausible to think that accumulation of unprocessed OPTM adducts can occur and may sensitize the immune system leading to the production of harmful cross-reacting antibodies to both OPTM adducts and bacterial antigens. Candidates for cross-reacting bacterial epitopes which have been proposed so far are phosphocholine (PC) and cardiolipin. It can be possible that more cross-reacting antigens will be discovered in the future, however, given that specific immunological tolerance to PC and cardiolipin exists under usual circumstances and given that the breakdown of the tolerance to commensal bacteria is the principle pathogenesis of IBD, PC and cardiolipin are more than enough to be considered as the direct causative agents.

The regulation of immunological tolerance may not be so simple that the increase of the untreated OPTM adducts can easily breakdown the tolerance. It is also known,

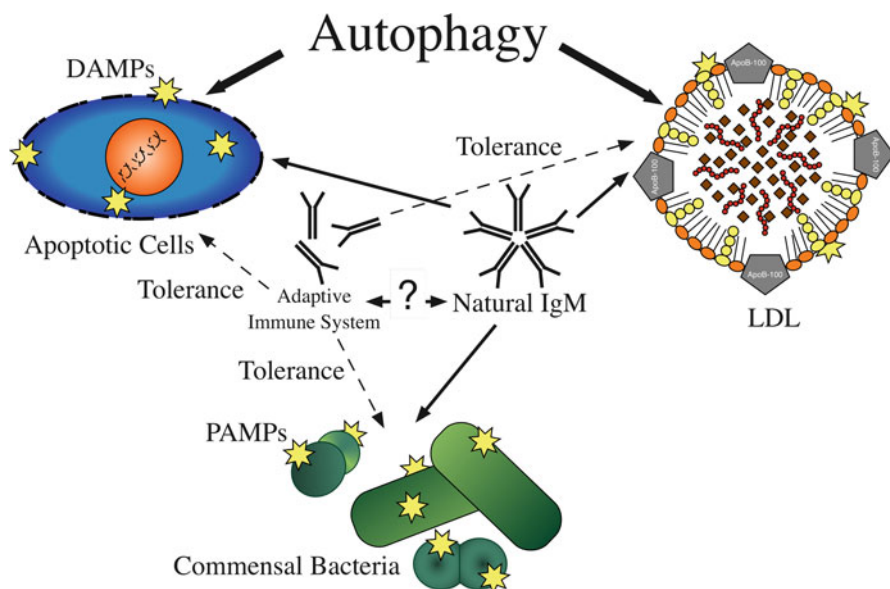


Fig. 2.2 Homeostasis of immune responses in healthy individual. This figure shows schematic expression of homeostasis of immune responses in healthy individual. Biomolecules (such as LDL) undergoes oxidative stress-induced post-translational modification (OPTM) and certain epitopes are converted to unusual forms. Such unusual epitopes (adduct) share molecular similarity with damage (Danger) associated molecular patterns (DAMPs) on apoptotic cells and pathogen associated molecular patterns (PAMPs) on commensal bacteria. This shared epitope is recognized by natural antibody whose subclass is IgM. Oxidatively modified proteins and apoptic cells are removed from cells and tissues by autophagy; therefore, the epitope does not evoke severe immune responses. At the same time, specific immunological tolerance exists towards commensal bacteria of our body

however, that both of those cross-reacting antigens, i.e. OPTM adducts and bacterial epitopes, are recognized by natural antibody IgM, which is one of the components of innate immunity. Therefore, there can be yet unknown regulatory mechanisms bridging innate and adaptive immune systems. This void in research further strengthens the need for future investigations.

2.3.6 *Post-translational Protein Oxidization in PD*

In case of PD, similar mechanisms which is underlying in the pathogenesis of IBD can be involved and this can be the reason why these two diseases show resembling natural history as described above (Fig. 2.4). Additionally, a noteworthy paper was published this year on the involvement of OPTM in PD. Turunen et al. [62] demonstrated that natural antibody IgM that is specific to one of the OPTM adducts,

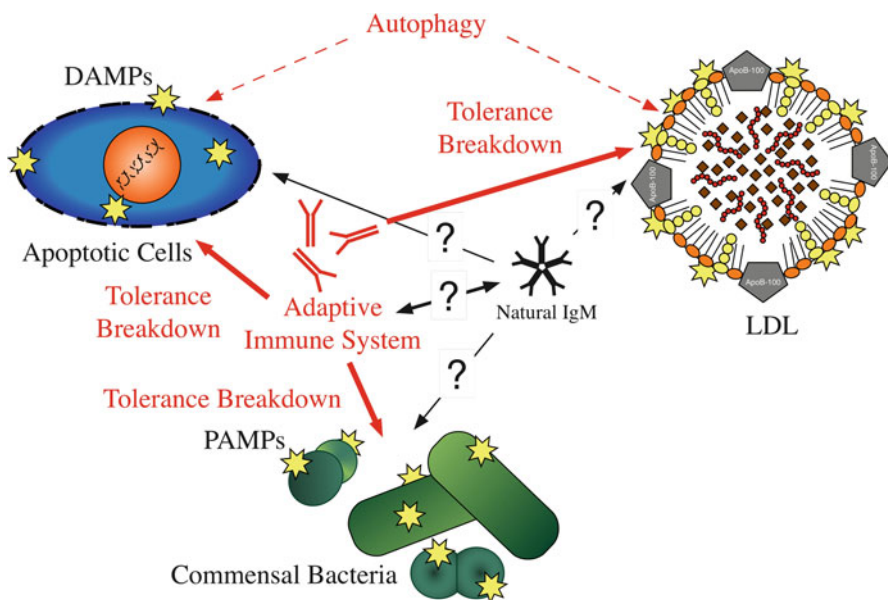


Fig. 2.3 Hypothetic involvement of OPTM in the pathogenesis of IBD. This figure shows schematic expression of the involvement of OPTM in IBD. Due to the predisposed factors, the function of autophagy declines in susceptible individuals. This leads to the accumulations of untreated modified proteins and apoptotic cells which further result in increase of shared epitope. This increase alters systemic immunological status and induce strong systemic immune response specific to the shared epitope. This immune response shows cross-reactivity with PAMPs on commensal bacteria and causes breakdown of immunological tolerance against commensal bacteria which is a proposed pathogenesis of IBD

malondialdehyde-oxidated molecule (MDA molecule) on oxidated LDL (OxLDL) has cross-reactivity against gingipain on *P. gingivalis* which is a major causative microbial agent of PD.

Although the aim of the authors was to suggest the potential roles of a natural antibody specific to this shared epitope in both atherosclerosis and PD, this paper provided a lot of implications to the pathogenesis of PD.

First of all, their data demonstrated that immunization with killed *P. gingivalis* whole cell or various fragments of gingipain was protective to atherogenesis, probably through diminishing uptake of OxLDL by macrophages. This result went against many previous publications reporting the positive relationship between atherosclerosis and *P. gingivalis* infection, because specific antibodies against major pathogenic factors are usually induced after infections. It is of note that the antibody subclass induced by *P. gingivalis* immunization was IgM class and gingipain-specific IgG antibody was not developed in either wild-type C57BL/6 or LDLR^{-/-} mice. This finding is conflicting against the previous papers demonstrating that IgG class antibody could be induced by the immunization of gingipain [63–66]; however, it must be noted that either incomplete or complete Freund's adjuvant was used in the immunization in each of those papers, while adjuvant was not used in Turunen

Finally and most importantly, it is possible that results from Turunen et al.'s paper are indicating that specific immunological tolerance against gingipain on *P. gingivalis* can be broken down when an OPTM adduct that shows molecular mimicry to gingipain increases and starts inducing cross-reacting antibodies. The induced gingipain-specific antibodies can be exudated into gingival crevicular fluid and can initiate aberrant immune responses leading to chronic inflammation.

As briefly mentioned earlier, two bacterial epitopes are known to show molecular mimicry with OPTM adducts. They are phosphocholine (PC) and cardiolipin. Many bacteria express epitopes containing PC, including approximately 30 % of the strains colonizing the oral cavity such as *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Streptococcus sanguis* [62, 67, 68]. Bacterial strains can be classified as PC-bearing or devoid of PC according to their ability to incorporate choline from the culture medium [69].

The significance of Turunen et al.'s paper is that they discovered a novel cross-reacting epitope on the major periodontopathic bacteria *P. gingivalis*. Interestingly, some *P. gingivalis* strain is devoid of PC [69], but bears this new cross-reacting antigen. This feature may make *P. gingivalis* a unique candidate causative agent among many oral commensal bacteria. This paper is providing a strong rationale to the involvement of protein oxidative modification in the pathogenesis of PD. Further investigations are expected if other putative periodontopathic bacteria have their own antigens which have molecular mimicry with OPTM adducts.

2.4 Conclusions

Association of protein oxidative modification in the pathogenesis of PD has been proposed many years. A new hypothesis of the potential mechanism explaining the association was discussed: it is the breakdown of immunological tolerance against the causative bacteria, which are commensal bacteria. This hypothesis can be applicable to both IBD and PD because both diseases can be caused by the breakdown of immunological tolerance to commensal bacteria. The breakdown of immunological tolerance to self-antigens caused by the accumulation of untreated OPTM adducts have been proposed in autoimmune diseases or chronic inflammatory diseases and the proposed underlying mechanism is molecular mimics between self-antigens and OPTM adducts.

To our knowledge, this review article is the first one in which the above concept was introduced to IBD and PD research and discussed.

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Studies on Periodontal Disease

Ekuni, D.; Battino, M.; Tomofuji, T.; Putnins, E.E. (Eds.)

2014, XII, 370 p. 37 illus., 20 illus. in color., Hardcover

ISBN: 978-1-4614-9556-7

A product of Humana Press