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Role of Oxidative Stress in the Cardiovascular Complications of Kawasaki Disease

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Abstract

Kawasaki disease (KD) is a rare and often undiagnosed disease, at least in the western countries. Although its etiology remains unidentified, epidemiological features point to the role of infection and genetic predisposition. KD is characterized by an inflammatory acute febrile vasculitis. Coronary artery involvement is the most important complication of KD and may cause significant coronary stenosis resulting in ischemic heart disease. It has been demonstrated that the major risks in KD progression are the endothelial dysfunction and that systemic oxidative stress together with premature aging of red blood cells and alteration of platelet homeostasis, could play a critical role in the cardiovascular complications associated with KD. This chapter will focus on the role of oxidative stress in endothelial damage and on circulating blood cells of KD patients.

Keywords: etiology, oxidative stress, inflammation, biomarkers,
red blood cells, platelets

1. Introduction

Kawasaki disease (KD) is an inflammatory acute febrile vasculitis that can also lead to coronary artery weakening, aneurysm formation, and myocardial infarction. The incidence of this disease varies considerably between ethnic groups: in Asians are up to 20 times higher than Caucasians. KD is most prominently recognized in Japan, Korea, and Taiwan, reflecting increased genetic susceptibility among Asian populations. The highest incidence is reported in Japan: about 90 per 100,000 [1, 2]. Although nearly 50 years have passed from the first description, the etiology of KD remains a mystery. Since the incidence of the disease is high among Japanese people, it can be speculated that this people may have some sort of genetic characteristic that leaves them susceptible to KD. In addition, both clinical and epidemiological findings strongly suggest that some infectious agent or bacterial super-antigenic toxin can play a pathogenetic role in genetically susceptible individuals [3]. Despite KD patients in the acute phase receive high-dose intravenous

immunoglobulin (IVIG) and aspirin therapy, up to 5% of those affected will develop coronary aneurysms, predisposing them to thrombotic complications that could result in atherosclerosis, myocardial infarction, and/or death [4]. In fact, risk factors for the development of atherosclerosis such as C-reactive protein (CRP), oxidative stress (OS), and inflammatory cytokines, are increased in the acute phase of KD [5]. Moreover, in the acute phase of the disease, often patients undergo thrombocytosis that can exert a pathogenic role in the cardiovascular complications that characterize KD. However, in KD progression, the major risk is endothelial injury and coronary artery weakening, favoring the formation of aneurysms in 1:5 untreated children with KD as well as myocardial infarction, ischemic heart, and sudden death [6]. OS linked to inflammation that characterizes KD disease, has recently been included among the potentially useful diagnostic biomarkers in the vasculature of KD [7]. Several lines of evidence suggest that in KD patients, systemic OS may promote: (i) endothelial dysfunction through increased production of oxygen- and nitrogen-derived species (ROS/RNS); (ii) alter red blood cell (RBC) homeostasis, resulting in a sort of premature aging in these circulating cells that could lead to anemia and formation of blood clots; and (iii) stimulate platelet functions and defective platelet apoptosis program, resulting in thrombocytosis that can exert a pathogenetic role in the cardiovascular complications occurring in KD [8].

2. Kawasaki disease etiology

The etiology of KD remains one of the major mysteries in the field of Pediatrics, and no specific biological markers for diagnostic testing have been characterized to date. A large body of clinical, epidemiologic, immunologic, pathologic, and ultrastructural evidence suggests that environmental factors or infectious agents induce an intense inflammatory host response in genetically susceptible individuals [3]. The clinical findings of conjunctival injection, oral and pharyngeal erythema, cervical adenopathy, and rash, observed in patients with KD, are very similar to those observed in other pediatric infections acquired by the respiratory route.

2.1 Infections

Even if not confirmed, many published reports implicate a number of bacterial or viral pathogens such as *Staphylococcus*, *Streptococcus*, Adenovirus, human herpes virus 6 (HHV-6), Epstein Barr virus (HBV), human T-lymphotropic virus (HTLV), coronavirus and human bocavirus (HBoV) [9–19]. *Staphylococci and Streptococci* release exotoxins, known as super-antigens that promote the activation of a large numbers of T helper (Th) cells (5–20% of T cell clones) leading to an extensive immunological reaction [20]. Matsubara and collaborators state that toxic shock syndrome toxin-1 (TSST-1), Streptococcal Pyogenic Exotoxin A or C (SPEA or SPEC), and Staphylococcal Enterotoxin A or B (SEA or SEB) may act as super-antigens that could stimulate the immune system and result in KD [21]. However, despite an increase in: (i) anti-streptococcal SPEC antibodies in the sera of KD patients in acute phase [22] and (ii) anti-SPEC and -SPEA IgM found in the first few weeks following the illness [21], no significant differences in super-antigen antibody were found from some serological studies.

2.2 Immune dysregulation

Most investigators believe that derangement of the immune system and functional disorder of Th cells are the primary pathophysiologic features in patients with
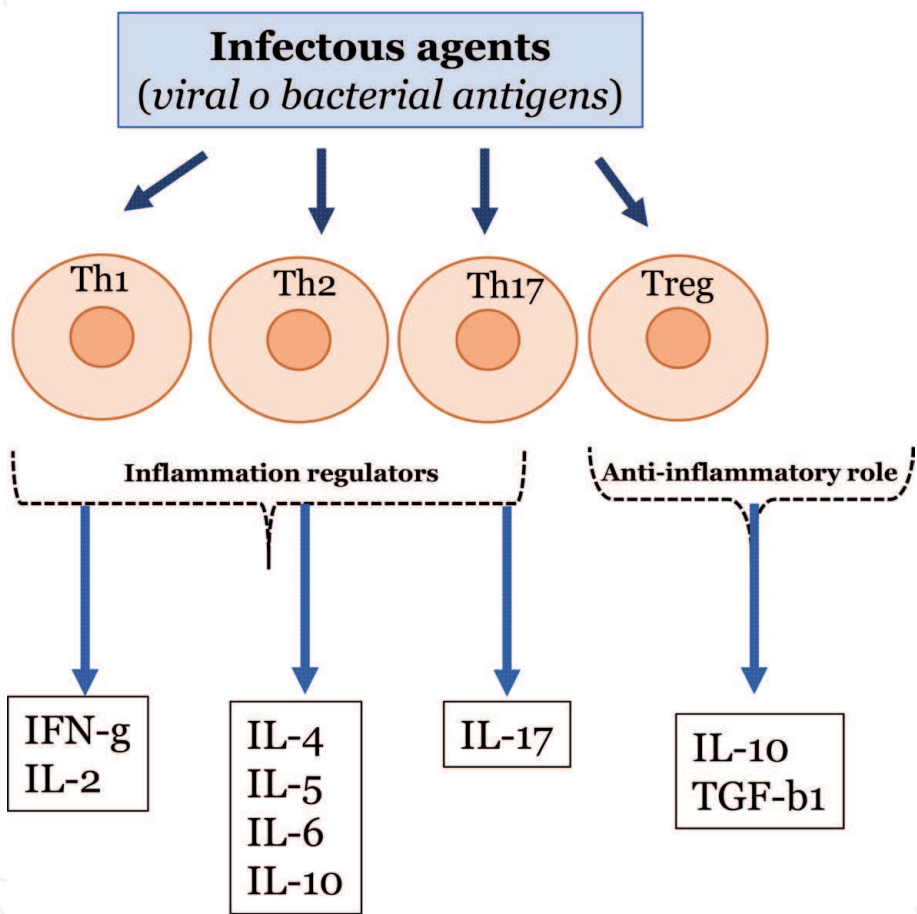
KD [23]. Data analyses for KD show that abnormal immune responses to infectious agents play key roles in disease initiation. It has been reported that, in the acute phase of KD, viral or bacterial super-antigens act by binding to the Vβ region of the T cell receptor inducing a widespread immunological response and resulting in the release of pro-inflammatory cytokines such as tumor necrosis factor (TNF) α, interleukin (IL) 1β, 6, 8, interferon (IFN) γ, and chemokines, such as monocyte chemotactic protein-1 (MCP-1) [3, 24]. In fact, it has been found that serum levels of some cytokines, such as IL-6, IL-20, TNF-α, and IFN-γ increase significantly before IVIG treatment and that levels of IL-6, IL-10, and IFN-γ decreased rapidly after treatment [25]. Moreover, studies in a murine systemic vasculitis, induced by *Candida albicans* extract, emphasize a relationship between the development of vasculitis and the overexpression of pro-inflammatory cytokines, such as TNF-α and IL-6 [26, 27]. The activation of the immune system and the cascade of inflammatory factors are considered as important features of KD. In fact, Th cells, mononuclear cells, macrophages and plasma cells, with a smaller number of neutrophils, are observed in various organ tissues of fatal cases of acute KD [23]. When activated, T helper cells mainly differentiate into two functionally distinct subsets, Th1 and Th2 cells. Th1 cells play an important role in cellular immunity by secreting IL-2 and IFN-γ, while Th2 cells involve the development of antibody-producing B cells via the secretion of IL-4, IL-5, IL-6, and IL-10. Some of these cytokines play an important role in the progression from systemic activation of the immune system to local inflammation in coronary vessels. Recently, it has been demonstrated that KD patients may be non-responsive to IVIG when, after IVIG treatment, the serum levels of IL-6 and IL-10 decrease slowly and the levels of IL-4 and TNF-α increase [25]. Although activation of the immune system and production of various cytokines have both been reported in patients with KD, the role of T cells and the functional state of Th1 and Th2 cells in KD are still not fully understood. Moreover, an imbalance between the line Th 17 (Th17) and regulatory T (Treg) cells has been described in the peripheral blood from patients with KD [28]. Th17 cells have been identified as inflammation regulators via production of distinct cytokines, such as interleukin IL-17. Conversely, to the Treg cells expressing FOXP3 has been attributed an anti-inflammatory role via production of anti-inflammatory cytokines, for example, IL-10 and TGF-β1 [29]. T helper cells involved in KD etiology are listed in **Figure 1** and **Table 1**.

2.3 Genetics

For decades, researchers attempted to identify candidate genes conferring susceptibility to the KD. In particular, studies on genes related to innate and acquired immune functions or to vascular remodeling, have been conducted [30]. Genes for analyses were selected based on the information of their known function or role in the disease pathophysiology. Initial genetic studies were focused on human leukocyte antigen (HLA) genes, located at chromosome 6p21.3, that encode the protein on the cell-surface antigen-presenting proteins, involved in the regulation of the immune system. The roles of HLA genes have been investigated in several immune-mediated vascular diseases, including KD. The results of such studies vary depending on the ethnic group studied. A recent genome-wide association study demonstrated the significant association of HLA class II region (HLA-DQB2-DOB) with KD in a Japanese population [31]. A genome-wide association study conducted in a Korean population demonstrated a significant association with KD of the HLA class I locus that contains the HLA-B and HLA-C genes [32]. These studies suggest that either HLA class I or class II may be associated with KD and play a role in KD pathogenesis. Several reports show associations between

KD and specific HLA genotypes including HLA-B54 in a Japanese population [33], HLA-B51 in Caucasian populations [34], HLA-B35, -B75, and -Cw09 in Korean [35], and the major histocompatibility complex class I chain-related gene A (MICA) genes in southern Chinese [36]. Genome-wide association studies (GWASs) have identified several susceptibility genes associated with KD, including *CD40L*, *HLA-E*, *BLK*, and *FCGR2A* [37–40]. CD40L gene, located on Xq26, is known to induce endothelial cells to produce cell adhesion molecules and chemokines. Its expression has been found elevated on CD4+ Th cells and platelets during the acute-phase KD and in KD patients with coronary artery lesions (CALs)

Figure 1.
T helper cells involved in KD etiology. In the acute phase of Kawasaki disease, viral or bacterial super-antigens induce immunological response resulting in the release of cytokines. Th cells are regulators of inflammation. T helper 1 cells secrete interferon g (IFN-g) and interleukin 2 (IL-2); T helper 2 cells secrete interleukin 4, 5, 6, and 10; T helper 17 cells secrete interleukin 17 (IL-17). Conversely, regulatory T cells (Treg) have an anti-inflammatory role via production of interleukin 10 (IL-10) and growth factor-beta (TGF-b).

T helper cells	Functions
Th1	Regulate cellular immunity by secreting IL-2 and IFN-γ
Th2	Regulate humoral immunity by secreting IL-4, IL-5, IL-6, and IL-10
Th17	Regulate inflammation by secreting IL-17
Treg	Anti-inflammatory role by the release of IL-10 and TGF-β1

Treg, regulatory T cells.

Table 1.
Immune dysregulation in KD: role of T helper cells.

[41]. HLA-E is a known ligand of CD94/natural killer cell receptor group 2-A (NKG2-A) and CD94/NKG2-C, which are expressed on natural killer cells [38]. Recent studies have shown that HLA-E has regulatory functions in both the innate and adaptive immune responses and that may have important implications in the pathogenesis of immune-mediated vascular diseases [42]. *BLK* is a Src family tyrosine kinase expressed primarily in the B-cell lineage and located on chromosome 8p22-23. During the acute and convalescent stages of KD, *BLK* expression correlates with the percentage of B cells in the peripheral blood mononuclear cells. Importantly, a decreased *BLK* expression in peripheral blood B cells may alter B cell function and predispose individuals to KD [43]. The *BLK* was significantly associated with KD susceptibility in Taiwanese and in Japanese populations [43]. *FCGR2A* gene is on chromosome 1q23 and encodes the FcγRIIA protein (CD32a), a member of a family of receptors for IgG (including the A, B, and C subunits of FcγRI and FcγRII and the A and B subunits of FcγRIII). This receptor is found on the surface of many immune cells, including natural killer cells, macrophages, and neutrophils, and it is involved in cellular activation and uptake of immune complexes [44]. The *FCGR2A* is associated with KD susceptibility in Korean and Asiatic populations [30]. Genes related to vasoactive or angiogenic molecules also can be considered as candidates for KD susceptibility or severity. Ohno and co-worker have shown that an up-regulation of vascular endothelial growth factor (VEGF) is involved in formation of coronary artery lesions (CALs) [45]. VEGF, expressed in various types of cells including leukocytes and vascular smooth muscle cells, binding to its receptor (VEGFR-1 and VEGFR-2) expressed on endothelial cells induces cell proliferation, survival, migration, and angiogenesis. Its ability to induce vascular hyper-permeability and chemotaxis of bone marrow-derived cells suggest significant roles of VEGF in inflammation [45]. Other candidate genes for KD are transforming growth factor-beta (TGF-β), because TGF-β-mediated T-cell activation and cardiovascular remodeling are important features of KD. This gene, located on chromosome 19q13.1, modulates the balance of pro-inflammatory/anti-inflammatory T cells through a complex set of interactions [46]. Genetic variations in the TGF-β pathway may lead to an imbalance of pro-inflammatory and regulatory T cells (Treg) by affecting the expression of the forkhead/winged helix transcription factor P3 (FOXP3) that is involved in the differentiation, function, and survival of CD4 + CD25+ regulatory T cells. Several studies demonstrated that in the peripheral circulation of KD patients, Treg cell numbers were reduced, and their function compromised [47]. Recently, in KD, 191 genes mainly implicated in inflammation and innate immune response and some signaling pathway such as platelet activation have been identified. Among these genes, *MAPK14* and *PHLPP1* were considered as the key functional genes that can distinguish KD from common infectious illness [48]. *MAPK14* is a gene that encodes p38α, a MAP kinases implicated in various cellular processes including proliferation, differentiation, transcription regulation, and development [49]. MPK14/P38 was found to significantly improve endothelial function and inflammation after vascular injury. *PHLPP1* encodes a protein that is a member of the Ser/Thr phosphatase family. Its upregulation in acute KD may reduce vascular injury by inactivating Akt and subsequent reducing the expression of NO [48]. Moreover, genetic polymorphisms of 1,4,5-trisphosphate 3-kinase C (ITPKC) and caspase 3 (CASP3) have been shown to associate with coronary artery lesions formation in both Japanese and Taiwanese populations of KD patients [50]. ITPKC is a gene located on chromosome 19q23 that acts as a negative regulator of T-cell activation. CASP3 is a gene located on chromosome 4q35, that is related to the apoptosis of immune cells [50]. Candidate genes in the KD etiology are listed in **Table 2**.

Candidate genes	Locus	Populations	Function
HLA-B54	6p21.3	Japanese	Regulation of the immune system
HLA-B51	6p21.3	Caucasian	Regulation of the immune system
HLA-B35	6p21.3	Corean	Regulation of the immune system
HLA-B75	6p21.3	Corean	Regulation of the immune system
HLA-E	6p21.3	Taiwanese	CAL formation
HLA-Cw09	6p21.3	Corean	Coronary complication
MICA	6p21.3	Southern Chinese	CAL formation
CD40L	Xq26	Taiwanese	CAL formation
BLK	8p22-23	Taiwanese and Japanese	Correlation with the % of B cells during KD
FCGR2A	1q23	Korean and Asiatic	Cellular activation and uptake of immune complexes
VEGF	6p12	Japanese	CAL formation
TGF-β	19q13.1	European	Modulates the balance of proinflammatory/anti-inflammatory T cells
MAPK14	6p21.31	Chinese	Autoimmunity-related vasculitis
PHLPP		Chinese	Reduce vascular injury
ITPKC	19q23	Taiwanese	Inactive T cells
CASP3	4q35	Taiwanese	Apoptosis in immune cells

HLA, human leukocyte antigen; MICA, major histocompatibility complex class I chain–related gene A; BLK, B-lymphoid tyrosine kinase; FCGR2A, Fc fragment of IgG receptor IIa; VEGF, vascular endothelial growth factor; TGF-β, transforming growth factor-beta; MAPK14, mitogen-activated protein kinase 14; PHLPP, PH domain leucine-rich repeat-containing protein phosphatase 1; ITPKC, 1,4,5-trisphosphate 3-kinase C; CASP3, caspase 3.

Table 2.
Candidate genes conferring susceptibility to the KD.

2.4 Environmental factors

Environmental factors, including socio-economic status and cultural habits in a society, affect the occurrence of infectious and autoimmune diseases. Recent studies suggest that environmental triggers, such as air pollution and extreme temperatures, may also serve as risk factors for KD [51]. Particulate matter and various gaseous pollutants, contained in the ambient air, have strong oxidizing property and the potential to induce KD through exaggerated inflammatory response, which is heavily involved in the pathophysiologic process of KD development [52]. Short-term exposure to air pollutants may damage endothelial cells, impair vascular function, stimulate systemic inflammation response, increase oxidative stress, and induce cardiac ischemia and repolarization abnormalities [52–54], consequently contributing to the development of KD. Moreover, from a time-stratified case-crossover study in Taiwan, evidence has been provided that exposure to ozone (O_3) may increase the risk of KD in children [55].

Recently, a study carried out on the Japanese population has found an association between higher household income, urbanization, and smaller family size at birth with increased KD incidence, which raises the hygiene hypothesis for the etiology of KD [56].

It has been reported that the human immune system and microbiota are trying to adapt to a changing environment. Gut microflora of infants were different

according to ethnic groups, and the changing environment factors from industrialization may affect the distribution of gut microflora in infants [57]. Thus, it is very possible that normal flora also adjusts to a changing environment. Presently, the majority of data has found that the composition of the gut microbiota in KD patients differs from healthy subjects. Lee and co-workers have hypothesized that the immune system should lose tolerance to a part of the resident intestinal flora and that environmental factors, that is, a Western lifestyle or improved public hygiene systems, could transform the commensal flora into a pathogen one, as observed in different gastrointestinal disorders [58].

3. Implication of systemic oxidative stress in KD

It has been recognized that a systemic pro-oxidant state associated with inflammation can play a key role in the pathogenesis and progression of KD [59]. In support to this theory, experimental evidences showed increased concentration of oxidative stress-related biomarkers such as ROS/RNS, malondialdehyde (MDA), protein 3-nitrotyrosine, asymmetric dimethylarginine (ADMA), and myeloperoxidase (MPO). ROS/RNS are chemical heterogeneous molecules that include radical species, such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radicals ($^{\bullet}OH$), and nitric oxide ($^{\bullet}NO$) and non-radical species such as hydrogen peroxide (H_2O_2) and peroxynitrite (the product of the fast reaction between $O_2^{\bullet-}$ and $^{\bullet}NO$). Peroxynitrite-mediated oxidation includes its direct reaction with several cellular targets (CO_2, hemoproteins, and thiols), as well as indirect reaction, CO_2-dependent oxidations mediated by strong oxidizing radicals, such as $^{\bullet}NO_2$ and carbonate radical ($CO_3^{\bullet-}$). The production of these oxidants is known to generate in blood a pro-oxidant status able to promote the occurrence of oxidative- and nitrative stress as well as redox imbalance leading to altered cell signaling and functions. These events may play a pathogenetic role in the cardiovascular complications often associated with KD [8]. As already mentioned, ROS/RNS generically can react with all the macromolecules of biological importance in cell and tissues, generating oxidative modification in lipids, DNA, and proteins that, in some cases, can be the footprint of the oxidant generated [60]. Malondialdehyde (MDA), the most investigated end-products of lipid peroxidation, is one of several low-molecular-weight end-products formed via the decomposition of certain primary and secondary lipid peroxidation products. It is a specific marker of omega-3 and omega-6 fatty acids peroxidation [61]. Increased serum levels of MDA were found in KD patients with coronary aneurysm associated with carotid intima-media thickening and stiffening [59]. Another marker of lipid peroxidation evaluated in KD patients is 8-isoprostaglandin F2α (8-iso-PG), a non-enzymatic oxidation product of arachidonic acid. Increased levels of 8-iso-PG have been measured in the urine from acute KD patients before IVIG therapy [62, 63]. Its increase reflects an enhanced endothelial dysfunction and correlates with cardiac dysfunction in acute KD [62]. Protein tyrosine nitration is an oxidative post-translational covalent modification of tyrosine residues consisting, in the addition of a nitro group ($-NO_2$) to the position 3, of the phenolic ring leading to the formation of 3-nitrotyrosine as an end-product [64]. It is a free-radical-mediated reaction induced by the one-electron oxidation of tyrosine residues to tyrosyl radical followed by its fast reaction with the nitrating agent $^{\bullet}NO_2$. In biological systems, 3-nitrotyrosine formation is mediated mainly by peroxynitrite-derived strong oxidants, such as $^{\bullet}OH$, $^{\bullet}NO_2$, $CO_3^{\bullet}$ [64]. In addition, 3-nitrotyrosine formation can be mediated by metals of heme-containing peroxidases in the presence of H_2O_2 and nitrite. The H_2O_2-genereted oxo-metal compounds (O = MnIV) and compounds I and II of heme-containing peroxidases, such as MPO, are highly heme oxidation

state complexes able to oxidize tyrosine to tyrosyl radical, which in the presence of $^{\bullet}NO_2$, generate 3-nitrotyrosine [65, 66]. Protein tyrosine nitration is considered a hallmark of the reactions involving $^{\bullet}NO$-derived oxidant, that is, peroxynitrite and $^{\bullet}NO_2$, able to dramatically affect protein structure and function. Indeed, the occurrence of this oxidative modification leads to a loss- (superoxide dismutase, prostacyclin synthase, etc.) or to a gain-of-function (cytochrome c, protein kinase, glutathione S-transferase, etc.) of key macromolecules able to affect cell homeostasis and fate [64]. The well-established association of protein tyrosine nitration to several pathologies, such as cardiovascular disease, neurodegeneration, inflammation and cancer has made this protein modification not only a biomarker of RNS-derived oxidative stress *in vivo*, but also a predictor of disease onset and progression. MPO is a pro-oxidant enzyme released by activated poly-morphonuclear leukocytes that can promote the pro-inflammatory state by inducing the formation of RNS, 3-nitrotyrosine, and lipid peroxidation [65]. Furthermore, it can promote a blood pro-coagulant state favoring the binding of oxidized lipoproteins to the specific receptor present on the surface of platelets [67]. In this regard, 3-nitrotyrosine and MPO could play a pathogenetic role in the cardiovascular complications of KD and could be considered as biomarkers of inflammation in this disease. Indeed, elevated MPO levels were detected in acute KD patients before IVIG treatment [8]. It has been recognized that a persistent OS and an excessive ROS production play an integral role in the endothelial and smooth muscle dysfunction leading to the risk of premature arteriosclerosis in KD patients [68]. A longer duration of fever is associated with higher risk of oxidative stress-induced endothelial dysfunction [68]. ADMA, produced following the catabolism of proteins containing methylated arginine residues, is an endogenous inhibitor of the enzyme nitric oxide synthase (NOS), regulating the nitric oxide bioavailability. Many disease states, including cardiovascular diseases and diabetes, are associated with increased plasma levels of ADMA [69]. This compound could therefore play a crucial role in the pathogenesis of diseases associated with endothelial dysfunction, so that it has been proposed as a biomarker for cardiovascular risk. In plasma from KD patients, low levels of ADMA were detected before IVIG treatment and associated with coronary abnormalities [8, 70]. Moreover, it has been suggested that a pro-oxidant blood status could alter RBC homeostasis [71]. RBCs, under physiological conditions, represent the major components of blood antioxidant capacity and the cells with higher resistance to oxidative stress [71]. They exert a scavenging activity with a particular regard for ROS and for the species derived from nitric oxide, often overproduced in inflamed tissues. In fact, crossing inflamed areas can contribute to detoxify ROS and RNS "protecting" cells (e.g. endothelial cells). In contrast, when they cross a tissue where an intense production of ROS occurs, they may accumulate oxidative damage and become a source of reactive species capable of modifying the behavior and fate of endothelial cells [72]. In KD patients, alterations of RBCs, typically associated with oxidative imbalance, have been detected [8]. In particular, increased ROS levels and reduced intracellular total thiol content were measured in RBCs from KD patients before treatment with IVIG and aspirin. In addition, the appearance of RBCs with alterations typically associated with premature aging (e.g. glycophorin A and CD47 expression) or eryptosis (e.g. clustering of band 3 and increase of phosphatidylserine externalization) was observed. Glycophorin A (GA) is a glycoprotein widely expressed at the RBC surface that is downregulated during senescence. CD47 is an integrin-associated protein. Known as thrombospondin receptor, it acts as marker of self. Band 3 is an ion exchanger involved in RBC adhesion to endothelium. Phosphatidylserine (PS) is a phospholipid normally localized to the inner leaflet of the plasma membrane. During cell remodeling, it is externalized to the outer leaflet leading to RBC aging

and death (eryptosis). Importantly, the appearance of aged and eryptotic RBCs in KD patients correlates with some clinical evaluations. In fact, it has been found that during the first 5 days of hospitalization, the number of RBCs, hemoglobin, mean corpuscular value, and hematocrit decrease significantly [8]. In addition, premature aging of RBCs, and their consequent removal from circulation, might be a risk factor for anemia: condition that can be found in KD patients. Furthermore, it has
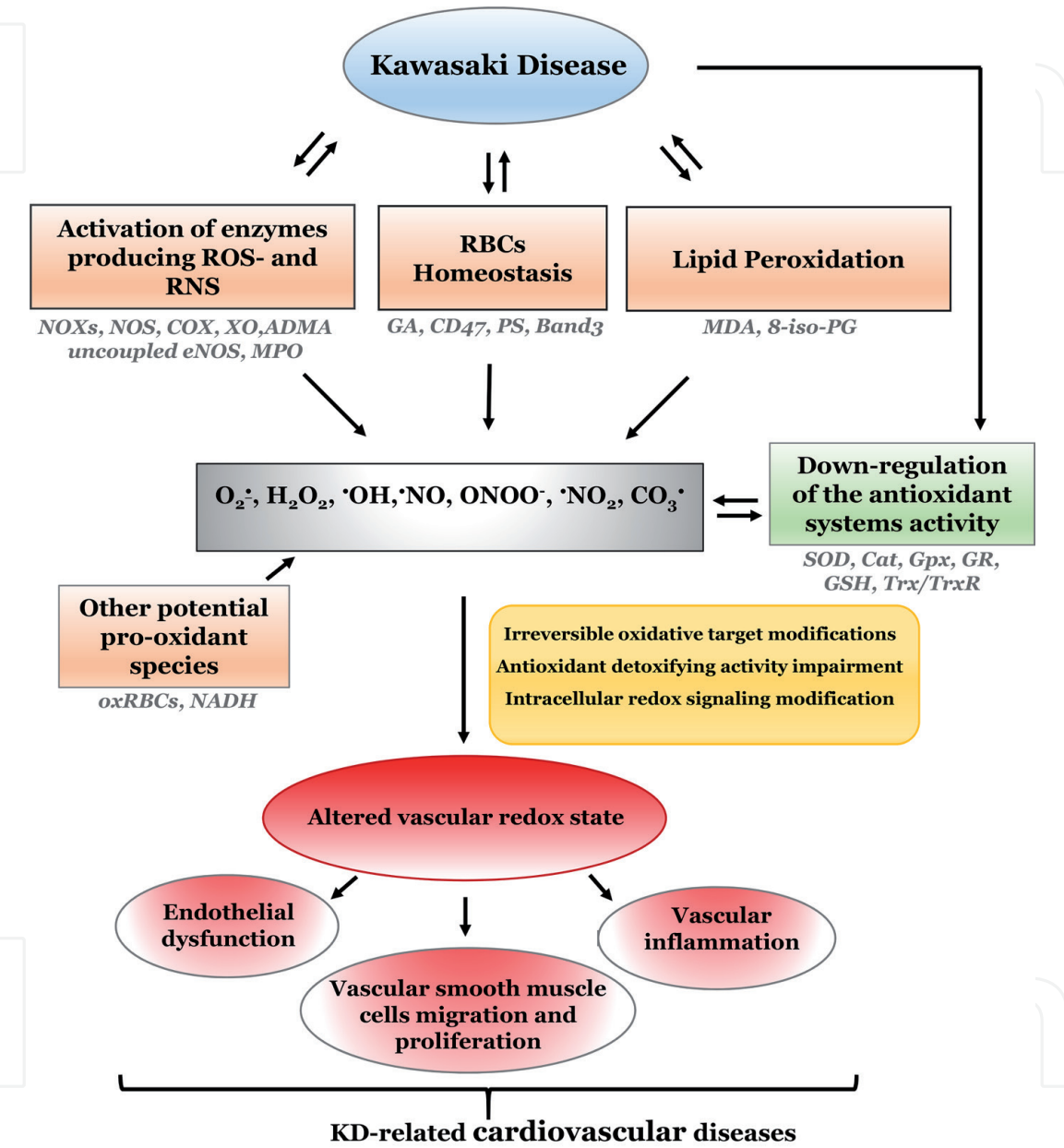
Figure 2.
Oxidative stress and vascular implications in Kawasaki disease. The Kawasaki disease (KD) is characterized by acute inflammation that has tissue oxidative stress as hallmark. This condition boosts the increase of reactive oxygen (ROS) and nitrogen (RNS) species formation in tissues and in the vasculature through the activation of the related producing enzymes in the cytosol. The cytosolic enzymes include the different isoforms of NADPH oxidase (NOXs), nitric oxide synthase (NOS), cyclooxygenase (COX), xanthine oxidase (OX), asymmetric dimethylarginine (ADMA), uncoupled endothelial NOS (eNOS), and myeloperoxidase (MPO). Other potential sources of ROS and RNS are the oxidized RBCs (oxRBCs) and lipid peroxidation. The Kawasaki disease is also characterized by the down-regulation of the antioxidant systems, including the depletion of GSH concentration and the decrease in the activity of the detoxifying enzymes, such as superoxide dismutase (SOD), catalase (Cat) glutathione peroxidase (Gpx), glutathione reductase (GR) and the couple constituted by thioredoxin (Trx) and thioredoxin reductase (TrxR). These conditions result in the irreversible accumulation of oxidation products in proteins, lipids, and sugars, which allow to the impairment of the intracellular redox signaling and detrimentally affect vascular biology by promoting vascular inflammation, endothelial dysfunction, and vascular remodeling. These alterations underlie the typical KD-associated cardiovascular complications, such as coronary artery weakening, aneurysm formation, and myocardial infarction.

been hypothesized that in KD patients, oxidative stress can alter platelet functions and platelet apoptosis program resulting in thrombocytosis that can exert a pathogenetic role in the cardiovascular complications [47]. This hypothesis is supported by the detection of markers of platelet activation, such as P-selectin shedding and PS externalization. P-selectin is a cell-adhesion molecule constitutively expressed in the α-granules of resting platelet. It translocates at the surface during platelet activation and subsequently released by a shedding phenomenon. Its release modulates leucocyte adhesion to both platelets and endothelial cells during inflammatory responses and thrombus formation [73]. PS externalization in platelets is usually associated with a sort of programmed cell death and correlated with their hyper-activation. In KD patients, before treatment with IVIG and aspirin, two different sub-populations of platelets have been identified: (i) annexin V positive platelets, characterized by a decreased mitochondrial membrane potential and

Plasmatic biomarkers	Specificity	Clinical findings in KD
ROS/RNS ($O_2^{\bullet-}$, $^\bullet OH$, $^\bullet NO$, H_2O_2)	Generates in blood a pro-oxidant status	Increased levels
MDA	Specific marker of omega-3 and omega-6 fatty acids peroxidation	Increased levels
Protein 3-nitrotyrosine	End-product of modification of tyrosine residues	Increased levels
ADMA	Endogenous inhibitor of the endothelial NOS. Regulates the NO bioavailability	Decreased levels
MPO	Pro-oxidant enzyme that can promote the pro-inflammatory state	Increased levels
RBC biomarkers		
Glycophorin A	Glycoprotein downregulated during RBC senescence	Down-regulated
CD47	Thrombospondin receptor that acts as a marker of self	Down-regulated
Band 3	Ion exchanger involved in RBC adhesion to endothelium	Down-regulated
PS externalization	Phospholipid, marker of RBC aging and death when externalized to the outer leaflet of the plasma membrane	Increased percentage of RBCs with externalized PS
Platelet biomarkers		
P-selectin	A cell-adhesion molecule that modulates leucocyte adhesion to both platelets and endothelial cells during inflammatory responses and thrombus formation	Shedding
PS externalization and loss of mitochondrial membrane potential	Biomarkers of pro-coagulant platelets	Detected
Mitochondrial membrane hyperpolarization without PS externalization	Biomarkers of potentially pro-coagulant platelets	Detected

ROS/RNS, oxygen- and nitrogen-derived species; $O_2^\bullet$, superoxide anion; $^\bullet OH$, hydroxyl radicals; $^\bullet NO$, nitric oxide; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; ADMA, asymmetric dimethylarginine; MPO, myeloperoxidase; RBC, red blood cell; PS, phosphatidylserine; NOS, nitric oxide synthase.

Table 3.
Biomarkers of oxidative stress in KD.

defined as activated pro-coagulant platelets [74] and (ii) annexin V negative platelets, characterized by an increased mitochondrial membrane potential, prone to become pro-coagulant when in contact with adenosine diphosphate (ADP) and thromboxane, mediators normally released from activated platelets [75]. It has been hypothesized that in KD patients, activated pro-coagulant platelets could contribute to the increased thrombotic risk detected in these patients. Implication of oxidative stress in KD is depicted in **Figure 2**. Biomarkers of oxidative stress in KD patients are summarized in **Table 3**.

4. Conclusions

In this chapter, a complex framework of events contributing to the etiology of KD has been described. These include some type of bacterial or viral infection, genetic determinants, immune system as well as hematological alterations. Although epidemiological and clinical data suggest that KD may arise from an abnormal response to infectious diseases in genetically susceptible individuals, there are still many controversies about the etiology of KD. There is no agreement on KD-related infectious agents, and the immune mechanisms behind KD remaining only partially known. Only the basic research evaluating the pathogenic mechanisms of this disease will probably find new targets for identifying disease-modifying agents or therapies that are more specific. Moreover, in this chapter, we provided new lines of evidence supporting the hypothesis that systemic oxidative stress together with premature aging of RBCs and platelets could play a critical role in the cardiovascular risk observed in patients with KD.

Abbreviations

ADMA	asymmetric dimethylarginine
ADP	adenosine diphosphate
CALs	coronary artery lesions
CASP3	caspase 3
$CO_3^{\bullet}$	carbonate radical
CRP	C-reactive protein
GA	glycophorin A
GWASs	genome-wide association studies
H_2O_2	hydrogen peroxide
HBoV	coronavirus and human bocavirus
HBV	Epstein Barr virus
HHV-6	human herpes virus 6
HLA	human leukocyte antigen
HTLV	human T-lymphotropic virus
IFN-γ	Interferon γ
ITPKC	1,4,5-trisphosphate 3-kinase C
IVIG	intravenous immunoglobulin
KD	Kawasaki disease
MCP-1	monocyte chemotactic protein-1
MDA	malondialdehyde
MICA	major histocompatibility complex class I chain-related gene A
MPO	myeloperoxidase
NKG2-A	natural killer cell receptor group 2-A
$^{\bullet}NO$	nitric oxide

NOS	nitric oxide synthase
$O_2^{\bullet-}$	superoxide anion
$^{\bullet}OH$	hydroxyl radicals
OS	oxidative stress
PS	phosphatidylserine
RBC	red blood cell
RNS	reactive nitrogen species
ROS	reactive oxygen species
SEA	Staphylococcal Enterotoxin A
SEB	Staphylococcal Enterotoxin B
SPEA	Streptococcal Pyogenic Exotoxin A
SPEC	Streptococcal Pyogenic Exotoxin C
TGF-β	transforming growth factor-beta
TNF-α	tumor necrosis factor α
Treg	regulatory T cells
TSST-1	toxic shock syndrome toxin-1
VEGF	vascular endothelial growth factor

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