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The Role of Neutrophil Activation in the Pathogenesis of Kawasaki Disease

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Abstract

In the acute phase of Kawasaki disease (KD), there is an increase in the number of circulating neutrophils, including toxic neutrophils, which are caused by a morphological change. The functions of the neutrophils are also activated, leading to the excessive production of reactive oxygen species and elastase, which may induce endothelial cell (EC) injury. Histological findings demonstrate that numerous neutrophils infiltrate into coronary artery lesions (CALs) during the early phase of KD. Thus, it is suggested that neutrophil-mediated EC injury may be involved in the formation of CALs in KD patients. In this review, we focus on the role of neutrophil activation in the pathogenesis of KD vasculitis and discuss the methods of treatment for neutrophil-mediated EC injury.

Keywords: Kawasaki disease; Neutrophil; Coronary artery lesion; Endothelial cell

Introduction

Human polymorphonuclear neutrophils play a key role in the host defense by ingesting and destroying infectious agents. Neutrophils contain a number of proteases in their own granules; the proteases are also involved in the physiological processes of matrix proteolysis, which include intercellular migration, tissue remodeling, and repair [1,2]. However, if the activity of the proteases secreted from neutrophils is uncontrolled and excessive in conditions such as severe inflammatory disease, they can degrade the extracellular matrix, thus leading to the destruction of tissue. Neutrophil elastase, which is stored in an active form within the azurophilic granules, is an enzyme from the class of proteases that break down proteins. Since elastase is the strongest serine protease, this enzyme is reported to play the main role in the neutrophil-mediated injury of endothelial cells (ECs) [1]. EC injury mediated by activated neutrophils has been reported to occur in systemic

inflammatory response syndrome (SIRS), adult respiratory distress syndrome (ARDS) and multiple organ failure (MOF) [3].

Kawasaki disease (KD) is an acute febrile illness that predominantly affects infants and children [4]. This disease is well known to be a type of systemic vasculitis, and the most critical complication is the occurrence of a coronary artery lesion (CAL), which may lead to myocardial infarction [5]. Although high-dose intravenous immunoglobulin (IVIG) is the standard initial therapy for acute KD [6,7], approximately 10-20% of KD patients are resistant to IVIG therapy. IVIG-resistant patients are at higher risk of developing CAL than IVIG-responsive patients [6,7]. Approximately 3% of KD patients are reported to have cardiac sequelae, such as coronary dilatation and aneurysm [8]. Although KD is the primary cause of acquired heart disease in children in developed countries, the etiology remains unclear. KD patients have high levels of circulating proinflammatory cytokines and soluble adhesion molecules, indicating that the immune system is markedly activated in this disease [9]. In the acute phase of KD, circulating neutrophils are known to increase in number and are functionally activated, which causes the excessive production of reactive oxygen species (ROS) [10] and elastase [11]. Furthermore, the elastase levels in the acute phase of KD are reported to be higher than in the acute phase of sepsis [11]. Thus, it is suggested that neutrophil-mediated EC injury may contribute to the formation of CALs in patients with KD. In the present review, we focus on the role of neutrophil activation in the pathogenesis of KD vasculitis and discuss the therapeutic methods for treating neutrophil-mediated EC injury.

The increased number of neutrophils and the morphological changes of neutrophils in acute KD

In the acute phase of KD, the white blood cell count is elevated and consists mainly of polymorphonuclear neutrophils with a shift to the left. Morphologically, many toxic neutrophils, which are characterized by cytotoxic vacuolization, swelling and toxic granulation with or without Döhle bodies, are seen in the circulation [12,13]. Furthermore, the percentage of toxic neutrophils in the acute phase of KD tends to be higher in patients with CAL than in those without CAL [12]. Kobayashi et

al. reported a risk-scoring system for predicting IVIG resistance, in which the percentage of neutrophils ($\geq 80\%$) is included as a factor [14]. Recently, we reported that an increased neutrophil-to-lymphocyte ratio can independently predict IVIG resistance in KD patients [15,16]. Thus, it is suggested that neutrophilia with toxic neutrophils may be a marker that reflects the severity of KD.

The role of neutrophil activation in the pathogenesis of KD vasculitis

Activated neutrophil-mediated EC injury in KD vasculitis: Histologically, the edematous changes of KD start in the media on the sixth to eighth day of illness, and inflammatory cells infiltrate into the intima and adventitia, forming panvasculitis across all layers of the vessel wall, by the tenth day of illness [17]. Takahashi et al. demonstrated the infiltration of numerous neutrophils (anti-elastase-positive neutrophils) in CALs during the early phase of KD (seventh to ninth day of illness), before the infiltration of macrophages and lymphocytes, suggesting that neutrophils are involved in the initial damage that occurs to the coronary arteries [18].

The function of neutrophils in the early stage of KD is reported to be enhanced with a marked increase in ROS [10]. The plasma levels of neutrophil elastase and myeloperoxidase (MPO) were reported to be increased in the acute phase of KD [11]. Furthermore, the plasma levels of neutrophil elastase were reported to be higher in patients with CAL than in those without CAL [19]. Biezeveld et al. reported that the plasma levels of neutrophil elastase remained high from the acute phase of KD throughout the convalescent phase (three months after the onset), suggesting that neutrophil activation may be sustained for longer than was previously assumed [20]. Thus, activated neutrophil-mediated EC injury is thought to be involved in the pathogenesis of KD vasculitis.

The up-regulation of the adhesion molecule expression of neutrophils in acute KD: Hamamichi et al. reported that circulating neutrophils abundantly express vascular endothelial growth factor (VEGF) in the acute phase of KD, suggesting that neutrophil-derived VEGF may contribute to the vascular injury and remodeling observed in KD vasculitis [21]. The CD11b expression of circulating neutrophils was increased in the acute phase of KD and was positively correlated with the serum levels of IL-6, IL-10, and granulocyte colony-stimulating factor (G-CSF) [22]. The expression of CD64 (Fc γ R) of neutrophils is reported to dramatically increase in the acute phase of KD and to decrease after IVIG infusion [23,24]. Since neutrophils primed *in vitro* with IFN- γ and G-CSF can induce the expression of CD64 [25,26], the up-regulation of CD64 may reflect the activation of neutrophils by inflammatory cytokines. The serum levels of G-CSF in IVIG-resistant patients were found to be significantly higher than those in IVIG-responsive patients, suggesting that the G-CSF stimulation of neutrophils may be an important risk factor for KD [27]. Circulating platelet-neutrophil aggregates are reported to be present at higher rates in patients with CAL than in those without CAL, suggesting that the platelet-neutrophil interaction may play a significant role in KD vasculitis by promoting the

migration and accumulation of neutrophils into vascular tissues [28].

The increased production of nitric oxide and nitric oxide synthase in acute KD: Nitric oxide (NO) is synthesized by nitric oxide synthase (NOS) and plays diverse roles in the physiology and pathophysiology of the cardiovascular system [29,30]. Although NO plays a vasoprotective role by inhibiting platelet aggregation, leukocyte adherence and vascular smooth muscle cell proliferation, the release of a large amount of NO can cause tissue damage and arterial wall degeneration. During the early stage of acute KD, it is reported that circulating neutrophils expressed both NO and NOS, and that the production level of inducible NOS (iNOS) in neutrophils was higher in patients with CAL than in those without CAL [31,32]. Thus, it is suggested that NO, which is synthesized by iNOS in neutrophils, may play an important role in the formation of CALs in KD vasculitis.

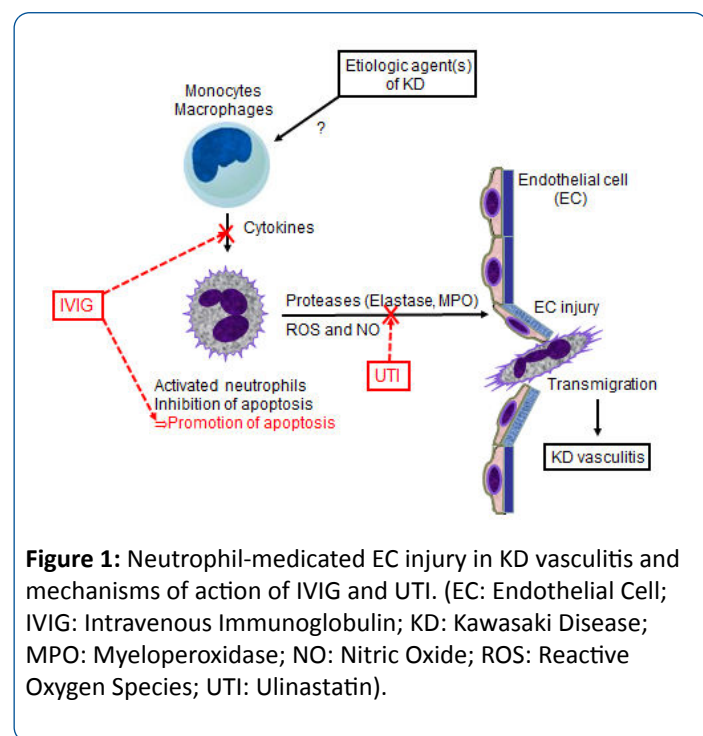
Delayed apoptosis of circulating neutrophils in acute KD: Human neutrophils are constitutively programmed to undergo apoptosis, and their life span is short. Since the prolonged survival of activated neutrophils has autotoxic potential due to the release of proteases and ROS, neutrophil apoptosis may represent a suitable mechanism for resolving inflammation. We previously reported that the apoptosis of neutrophils is inhibited during the acute phase of KD, and that an increase in the number of peripheral neutrophils may be associated with the delayed apoptosis of neutrophils [33]. Furthermore, IVIG can promote apoptosis in activated neutrophils *in vitro* by an oxygen-dependent pathway *via* Fc γ RII and III [34]. Actually, in KD patients, IVIG therapy is reported to decrease the number of circulating neutrophils by accelerating their apoptosis [35]. Thus, it is suggested that the prolongation of the life span of activated neutrophils in circulation may be involved in the pathogenesis of KD vasculitis, and that IVIG therapy may have a protective effect on activated neutrophil-mediated EC injury by inducing apoptosis in neutrophils.

The inhibitory effect of protease inhibitor on neutrophil-mediated EC injury in KD vasculitis: Although IVIG is the gold-standard therapy for acute KD, CAL formation cannot be completely prevented by IVIG alone [6,7]. Thus, it is believed that intensive initial therapy combined with primary IVIG and other anti-inflammatory drugs may reduce IVIG resistance and CAL formation in high-risk KD patients. Since the activated neutrophil-mediated EC injury is thought to be involved in the pathogenesis of KD vasculitis, the neutrophils may be a potential target of therapy in KD. Elastase is thought to be the primary agent of neutrophil-mediated EC injury [1]. Ulinastatin (urinary trypsin inhibitor, UTI), a serine protease inhibitor, inhibits neutrophil-mediated EC injury *in vitro*, by inactivating the extracellular elastase secreted from the neutrophils and suppressing the production of intracellular elastase in them [36]. UTI has been shown to have clinical applications in the treatment of inflammatory disorders such as pancreatitis, circulatory shock, septic shock, ARDS and disseminated intravascular coagulation (DIC) [37]. Recently, we reported that initial UTI therapy combined with IVIG reduced the proportion of KD patients requiring additional rescue treatment and the occurrence of CAL in comparison to IVIG alone [38]. Thus, UTI in

combination with IVIG may be a candidate for the initial treatment of KD.

Conclusion

A schematic illustration of neutrophil-mediated EC injury in KD vasculitis and the mechanisms of action of IVIG and UTI are shown in the **Figure 1**. In the acute phase of KD, circulating neutrophils are functionally activated by inflammatory mediators such as cytokines. The survival of the activated neutrophils, which produce excess amounts of proteases (elastase and MPO), ROS and NO, is prolonged because their apoptosis is delayed which may induce EC injury and neutrophil transmigration and contribute to the pathogenesis of KD vasculitis. Thus, the activated neutrophils may be a therapeutic target in KD. IVIG may not only inhibit the production of cytokines by monocytes and macrophages, but also promote the apoptosis of activated neutrophils. On the other hand, UTI may inactivate the elastase secreted from neutrophils and suppress the elastase production in them. Thus, it is suggested that both drugs protect ECs against neutrophil-mediated injury in KD vasculitis *via* different mechanisms. Since activated neutrophil-mediated EC injury is thought to be involved in the pathogenesis of KD, the clarification of its mechanism may lead to the improved treatment of this disease.



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Competing Interests

The authors have declared that no competing interests exist.

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