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Review Article

Recent advances in understanding the roles of blood platelets in the pathogenesis of allergic inflammation and bronchial asthma

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Abbreviations:
ADP, adenosine diphosphate; AERD, aspirin-exacerbated respiratory disease; cysLT, cysteinyl leukotriene; DAMPs, danger-associated molecular patterns; EETosis, eosinophil extracellular DNA trap cell death; ILC2, group 2 innate lymphoid cells; LPS, lipopolysaccharide; LT, leukotriene; NETs, neutrophil extracellular traps; PAF, platelet-activating factor; PAMPs, pathogen-associated molecular patterns; PF-4, platelet factor-4; PMP, platelet-derived microparticles; PMPs, platelet microbicidal proteins; PSGL-1, P-selectin glycoprotein ligand-1; tPA, tissue plasminogen activator; TLR, toll-like receptor

ABSTRACT

Platelets play an essential role in hemostasis to minimize blood loss due to traumatic injury. In addition, they contain various immune-associated molecules and contribute to immunological barrier formation at sites of vascular injury, thereby protecting against invading pathogens. Platelets are also crucially involved in development of allergic diseases, including bronchial asthma. Platelets in asthmatics are more activated than those in healthy individuals. By using a murine asthma model, platelets were shown to be actively involved in progression of the disease, including in airway eosinophilia and airway remodeling. In the asthmatic airway, pathological microvascular angiogenesis, a component of airway remodeling, is commonly observed, and the degree of abnormality is significantly associated with disease severity. Therefore, in order to repair the newly formed and structurally fragile blood vessels under inflammatory conditions, platelets may be continuously activated in asthmatics. Importantly, platelets constitutively express IL-33 protein, an alarmin cytokine that is essential for development of bronchial asthma. Meanwhile, the concept of development of allergic diseases has recently changed dramatically, and allergy researchers now share a belief in the centrality of epithelial barrier functions. In particular, IL-33 released from epithelial barrier tissue at sites of eczema can activate the antigen-non-specific innate immune system as an alarmin that is believed to be necessary for subsequent antigen-specific acquired immunological responses. From this perspective, we propose in this review a possible mechanism for how activated platelets act as an alarmin in development of bronchial asthma.

Introduction

Platelets are a kind of blood cell derived from bone marrow megakaryocytes and play essential roles in thrombosis, hemostasis and tissue repair. Vascular endothelial injury due to various causes such as trauma and ischemia leads to activation of platelets at the injured site, resulting in their adhesion, aggregation, release of granules and formation of platelet thrombi (primary hemostasis). Following the primary hemostasis, coagulation factors are sequentially activated, forming fibrin mesh (secondary hemostasis). To date, in addition to playing essential roles in hemostasis and subsequent tissue repair, platelets have been found to be crucially involved in various immune responses, in direct and indirect manners. Notably, clinical and experimental evidence

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demonstrates that platelets are actively involved in the pathogenesis of allergic diseases, including bronchial asthma.

In this review article, we focus on the roles of platelets in allergic inflammation and the pathogenesis of bronchial asthma, including the immunological implications of platelet activation in the asthmatic airway. We discuss the relationship between platelet functions *per se*, such as in hemostasis and tissue repair, and the latest concept regarding development of allergic diseases, which emphasizes epithelial barrier functions and the innate immune system. In particular, we propose a hypothesis concerning the mechanistic implications of platelet activation for the pathology of bronchial asthma.

Platelet functions in thrombosis, hemostasis and tissue repair – role of platelets as a physical barrier

When blood vessels are damaged and bleeding due to some cause such as trauma, it is necessary to induce hemostasis in order to promptly stop blood loss, and to repair damaged blood vessels and the surrounding tissues. Platelets are blood cells that play a central role in initiation of the hemostatic and tissue repair responses. Platelets are derived from cytoplasmic fragments of bone marrow megakaryocytes; they are approximately 2 μm in diameter and have no cell nucleus.[1,2] Approximately 10^{12} platelets are circulating in the blood of an adult human, and the lifespan of an individual platelet is about 10 days unless it is consumed in fibrin clot formation (whose process will be explained below). Therefore, an average of 10^{11} new platelets must be produced every day in a healthy adult to maintain a normal platelet count ($150–400 \times 10^9$/L of blood).[3]

Recruitment of platelets to a site of vascular injury is the first step in the process of hemostasis that plays a critical role in minimizing blood loss and forming a physical barrier against invading pathogens.[4] Under physiological conditions, circulating platelets remain in a quiescent state due to the inhibitory effects of both nitric oxide and prostaglandin I_2, which are constitutively produced by vascular endothelial cells.[5] At sites of bleeding, vasoconstriction of the injured blood vessels is the first response in order to limit blood loss. Subsequently, platelets adhere and accumulate on the damaged endothelium via von Willebrand factor. Activated platelets release the contents of their stored granules, which contain adenosine diphosphate (ADP), Ca^{2+}, thromboxane A2 (TXA_2), serotonin and platelet-activating factor (PAF), thereby further promoting platelet aggregation and formation of a platelet plug (primary hemostasis). Because the platelet plug formed during primary hemostasis is unstable and fragile, approximately a dozen coagulation factors that circulate in the bloodstream in an inactive state are quickly and sequentially activated in a so-called “coagulation cascade”, leading to fibrin mesh formation from inactive fibrinogen plasma protein. Hemostasis is completed when the fibrin mesh covers the platelet plug, creating a stable fibrin clot and holding it in place (secondary hemostasis).

Once their role in hemostasis is completed, clots must be broken up and removed, and the damaged tissue surrounding fibrin clots needs to be repaired. Intact vascular endothelial cells around clots produce tissue plasminogen activator (tPA). tPA catalyzes conversion of plasminogen to plasmin, the major enzyme responsible for clot breakdown (fibrinolysis).

It should be noted that, in addition to hemostatic factors, platelets contain various cell growth factors such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF), which play important roles in repairing and regenerating damaged tissue.[6] Thus, platelets are key players in all the processes triggered by bleeding, including primary hemostasis, secondary hemostasis, fibrinolysis and repair of damaged tissue, that both minimize blood loss and reinstate physical barriers to external substances.

Platelet functions in immune responses – role of platelets as an immunological barrier

Traumatic injuries cause bleeding and involve serious risk of invasion by foreign pathogens such as viruses and bacteria into the body. Therefore, in addition to physical barrier formation by hemostasis, a functioning immunological barrier to protect against invading pathogens must be formed as rapidly as possible at sites of injury. Indeed, in addition to their essential roles in thrombosis and hemostasis, platelets play important roles in assisting and modulating inflammatory reactions and immune responses in a wide range of ways, as described below.

Immune-associated molecules in platelets

Although platelets are anuclear cells derived from cytoplasmic fragments of bone marrow megakaryocytes, they contain various immune-associated molecules in intracellular granules such as α-granules and dense granules, as well as on their surface membrane.[7] For instance, P-selectin (CD62P), a cell adhesion molecule, is an integral membrane glycoprotein that is stored in α-granules in resting platelets.[8] Upon platelet activation by agonists such as thrombin and ADP, P-selectin is rapidly translocated onto the plasma membrane.[9] There, it plays an important role in initial recruitment of leukocytes, including neutrophils, monocytes and lymphocytes, to sites of injury via its ligand, P-selectin glycoprotein ligand-1 (PSGL-1), which is expressed on those cells.[10,11] Activated platelets also secrete various types of immune-associated molecules such as cytokines, chemokines, growth factors and lipid mediators in order to activate their interacting immune-cells and endothelial cells, and perhaps to modulate inflammatory processes at sites of injury. Regarding the roles of platelets as immune cells, please also see two excellent reviews.[6,7]

Platelets as killers of pathogens

As described above, platelets regulate inflammatory responses through immune cell recruitment and activation, but they can also kill pathogens. As an example, to defend against microbial invasion, platelets store various antimicrobial proteins called platelet microbicidal proteins (PMPs) in their α-granules, and thus platelets have direct antimicrobial functions.[12,13] PMPs include the CXC-chemokine family, such as CXCL4 (also known as platelet factor-4; PF-4)[14] and CXCL7 (also known as neutrophil-activating peptide-2; NAP-2).[15] With regard to platelets' functions in response to protozoan parasite infection, McMorran *et al.* demonstrated that platelets bind to malaria-infected red blood cells and can directly kill the parasites within.[16] This killing was found to be abrogated by aspirin and other platelet inhibitors. Furthermore, both thrombocytopenic and aspirin-treated mice were highly susceptible to death during erythrocytic infection by *Plasmodium chabaudi*, indicating that platelets are important in controlling malarial infection.

Platelets and neutrophils

Following microbial invasion of the body due to traumatic injury with bleeding, neutrophils are one of the first-responders among the various types of immune cells to quickly migrate toward sites of injury and eliminate invading pathogens by phagocytosis. Interestingly, in addition to their conventional phagocytosis function, neutrophils are able to capture and eliminate pathogens using a web-like “throwing implement” similar to a casting net, called

neutrophil extracellular traps (NETs). Brinkmann *et al.* first reported that activated neutrophils release nuclear DNA and intracytoplasmic antimicrobial proteins such as elastase and myeloperoxidase outside their cells by disrupting their cell membrane.[17] The process of releasing NETs is called NETosis, representing a type of cell death different from necrosis and apoptosis.[18,19] The pathogens captured by NETs tend to be more easily phagocytosed by neutrophils and macrophages, and NETs themselves contain various kinds of antimicrobial proteins (as described above), as a result of which they also have strong bactericidal action.[20] The latter study revealed that neutrophils can discriminate microbe size and selectively release NETs in response to large pathogens such as *Candida albicans* hyphae, but not in response to small yeasts or single bacteria.[21] Of note, platelets in the blood vessels play an important role in release of NETs from neutrophils. Platelets are activated by binding lipopolysaccharide (LPS), which is a structural component of the Gram-negative bacterial cell wall, through LPS specific receptor, Toll-like receptor-4 (TLR4), expressed on those cells. LPS-activated platelets express P-selectin and can transmit signals to its counterpart ligand, PSGL-1, on neutrophils, as a result of which platelet-activated neutrophils become further activated and release larger amounts of NETs extracellularly.[22,23] Indeed, NETs production requires platelet-neutrophil interactions, and it was inhibited by platelet depletion or disruption of platelet-neutrophil binding in a mouse sepsis model, resulting in diffusion and proliferation of circulating bacteria during sepsis.[18] Thus, platelets possess crucial functions that can efficiently protect against pathogen invasion at sites of injury through rapid (within less than an hour) control of NETs release from neutrophils.[18] In addition, eosinophils[24,25] and mast cells[26] are also known to be able to release extracellular DNA traps similar to NETs, but little is known regarding whether platelets can enhance formation of eosinophil- and/or mast cell-induced extracellular DNA traps. Although PAF itself cannot induce eosinophil extracellular DNA trap cell death (EETosis), it was shown to be able to induce EETosis when in combination with IL-5 or granulocyte macrophage colony-stimulating factor (GM-CSF).[24]

Taken together, platelets are intrinsically able to kill various pathogens in both direct and indirect manners by acting as an immunological barrier at sites of vascular injury.

Immunologic principles of allergic diseases – a new concept regarding development of allergic diseases and innate immunity

In this section, we digress from the topic of platelets to first describe the immunological principles of allergic diseases. After introducing the latest concepts regarding the mechanisms of development of allergic diseases, we will present an example that suggests an association between platelets and allergic inflammation.

Acquired immunity in allergic diseases

Allergic reaction are classically described as antigen-specific acquired immune responses elicited by B-cell-derived IgE antibodies. The initial response to a specific antigen creates an immunological memory (sensitization phase), and subsequent re-exposure to that pathogen leads to induction of an IgE-dependent response (reaction phase).[27] Upon re-exposure to the same sensitizing antigen, activated mast cells can release various chemical mediators, such as histamine, leukotrienes, PAF and prostaglandins, resulting in induction of various immediate allergic reactions (e.g., in asthmatic airway: induction of bronchial smooth muscle contraction, vascular permeability and mucus secretion). This antigen-specific immune system is called the “acquired immune system”, which is not yet developed at birth and requires a learning and memory process.

Innate immune system and alarmin

On the other hand, an immune response system called “innate immunity”, which is naturally present at birth and induced in an antigen-non-specific manner, has recently attracted attention as a definitive mechanism underlying the onset of allergic diseases. Innate immunity is a mechanism for quickly recognizing invading pathogens and alarmin (self-components that alert to danger; described later in detail), and it acts as the front-line of biological defense. The defense functions of neutrophils and/or platelets described above are also important players in innate immunity. The innate immune system is an evolutionarily ancient biological defense mechanism that is present in almost all multicellular organisms, including plants, whereas acquired immunity exists only in organisms more highly evolved than jawless vertebrates such as the lamprey. Its activation in response to pathogens or tissue injury is mediated via pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs), respectively.[28,29] Regarding the relationship between innate immunity and allergic disease, hitherto the innate immune system has been explained on the basis of a “hygiene hypothesis”. That is, at an early age viral and bacterial infections can act as adjuvants to stimulate toll-like receptors (TLRs) on dendritic cells. TLRs are a type of PAMPs receptor and can potentially inhibit development of allergic diseases by inducing inhaled-antigen-specific Th1 cells and regulatory T cells.[30] DAMPs, including high-mobility group box 1 protein (HMGB-1), heat-shock proteins (HSPs), uric acid and S100 proteins, are endogenous danger signals, also referred to as “alarmin”.[31,32] Alarmins are constitutively present in normal tissues and can be released at any time. Once tissues are accidentally damaged by trauma or an infection, alarmins are rapidly released from the damaged tissue to alert the immune system. A novel IL-1 family cytokine, IL-33, is an important alarmin that is constitutively expressed in the nuclei of epithelial and endothelial cells. IL-33 is released by necrotic cells after tissue injury and acts on its target cells (described later in detail).

A new concept regarding development of allergic diseases

It should be carefully noted that allergic diseases such as atopic dermatitis, hay fever and food allergy, as well as bronchial asthma, have common features. That is, these diseases all occur especially on the tissue surface/epidermis of the body due to excessive immune responses to foreign antigens that are harmless *per se*. During the past 10 years, our understanding of the onset of allergic diseases has changed dramatically. Today, researchers in the field of allergy share a new concept that emphasizes epithelial barrier functions, innate immunity and epicutaneous sensitization. One of the first reports serving as the impetus for this novel concept was a prospective birth cohort study by Lack *et al.* that demonstrated that low-dose exposure of inflamed skin of infants to peanut allergen, in the form of a skin care cream containing arachis oil, was significantly associated with increased risk of peanut allergy at 5 years of age.[33] Furthermore, in 2008, Lack proposed a “dual-allergen exposure hypothesis”, suggesting that sensitization to allergen occurs through environmental exposure of eczematous skin to allergen. That hypothesis also suggests that consumption of food allergens at an appropriate time and in adequate quantity can induce oral immune tolerance.[34] Thus, the dual-allergen exposure hypothesis may explain the significant association between early severe eczema in infancy and subsequent development of food

allergy. Indeed, loss-of-function mutations in the *filaggrin* gene, which is a key player in formation of the skin barrier, were strongly associated with atopic dermatitis.[35] Taken together, these reports have suggested that epidermal barrier dysfunction plays a key role in development of allergic diseases. Our group also reported that skin care by application of a moisturizer beginning from the neonatal period can effectively prevent subsequent development of atopic dermatitis.[36] Furthermore, our recent randomized, double-blind, placebo-controlled trial showed that for 6-month–old infants with eczema, introduction of heated egg, starting from a small dose in a stepwise manner, combined with optimal eczema treatment, efficiently prevented hen's egg allergy at 1 year of age.[37]

Although little is known about the precise immunological mechanisms underlying epithelial barrier dysfunction's role in antigen sensitization, antigen sensitization does not occur only due to physical invasion of allergens into the body as a result of epidermal destruction. Of note, endogenous factors released in eczema, such as “alarmins”, may be involved in establishment of antigen sensitization by modulating tissue-resident immune cells, including antigen-presenting cells.

IL-33: a novel innate immune cytokine that acts as an alarmin in development of allergic diseases

In this context, two major discoveries in the past decade provided important clues to the significance of the innate immune system in onset and progression of allergic diseases. First, several large-scale genome-wide association studies (GWAS) demonstrated that the genes for the alarmin cytokine, *IL-33*,[38] and its receptor, *ST2/IL1RL1*, are responsible for development of bronchial asthma.[39–42] Second, group 2 innate lymphoid cells (ILC2) were discovered to strongly evoke antigen-non-specific type 2 inflammation in response to IL-33.[43–46] ILCs are classified into 3 groups based on their cytokine production patterns (see a recent review of ILCs for details[47]). These findings suggest that the innate immune system including the IL-33/ST2 axis is strongly involved in development of bronchial asthma. To date, various immune cells—such as ILC2, mast cells, eosinophils, basophils, dendritic cells and Th2 cells—have been reported to be target cells of IL-33.[42] In particular, ILC2 were robustly activated by IL-33 alone or in combination with IL-2 to produce large amounts of such type 2 cytokines as IL-5 and IL-13.[43,44] Indeed, intranasal administration of IL-33 to mice significantly induced airway hyperresponsiveness, with increased type 2 airway inflammation, in the absence of the acquired immune system.[48] Our group also demonstrated that IL-33 is essential for papain-induced airway eosinophilia in mice, even in the absence of T cells, B cells and mast cells, which are major players in acquired immunity.[49] These results suggest that IL-33 is the crucial factor for induction of airway inflammation via the antigen-non-specific innate immune system. We previously demonstrated that human airway epithelial cells and microvascular endothelial cells in the lung constitutively express ST2/IL1RL1 protein and respond to IL-33 stimulation, resulting in rapid production of neutrophil-attracting CXCR2 chemokines, including IL-8 ([50]; and Matsuda *et al.*, unpublished data). Recently, we demonstrated that platelets constitutively express full-length IL-33 protein, which is the biologically active form of the cytokine,[51] and are crucially involved in papain-induced airway eosinophilic inflammation,[52] suggesting possible involvement of platelets in innate immune-mediated allergic diseases. Figure 1 depicts the putative roles of platelets and platelet-derived IL-33 as physical and immunological barriers in hemostasis.

Role of platelets in allergic inflammation and bronchial asthma

Platelets are known to be involved in the development of various kinds of disease, including cancer and inflammatory diseases, and platelet abnormalities in allergic states were already reported half a century ago.[53] Numerous subsequent clinical and experimental studies confirmed that platelets play important roles in the regulation of allergic inflammation.[54,55]

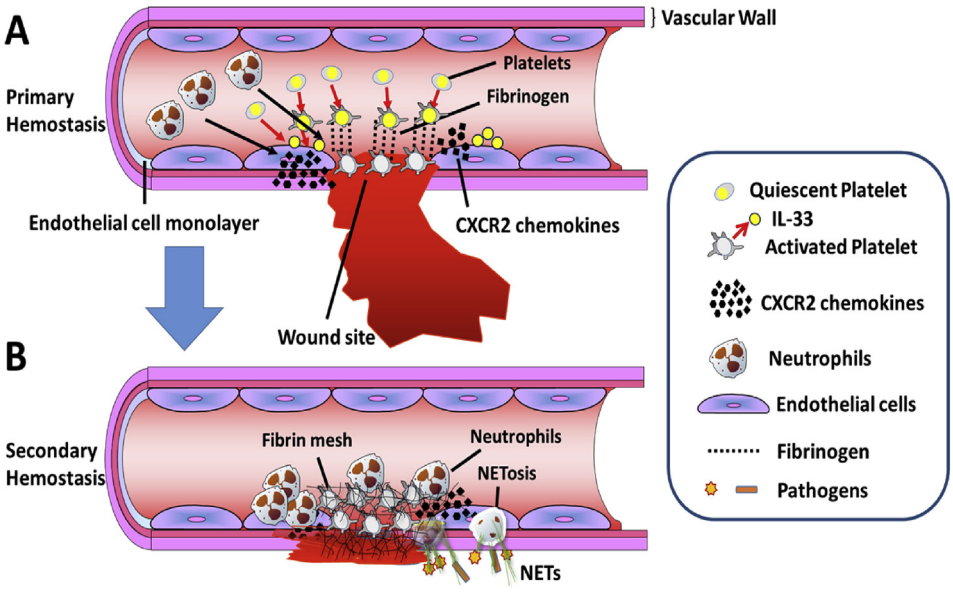

Fig. 1. Putative roles of platelets and platelet-derived IL-33 as physical and immunological barriers in hemostasis. (A) Vascular endothelial injury due to trauma, etc., causes platelets to be activated at sites of injury, leading to their adhesion, aggregation and release of granules, and formation of platelet thrombi (primary hemostasis). At that time, platelet-derived IL-33 can act on intact vascular endothelial cells near the injured site inducing large amounts of neutrophil-attracting CXCR2 chemokines such as IL-8. (B) Coagulation factors, which circulate in the bloodstream in an inactivated state, quickly and sequentially participate in the so-called “coagulation cascade”, resulting in fibrin mesh formation from inactive fibrinogen plasma protein (secondary hemostasis). Neutrophils are rapidly recruited to the lesion site by the CXCR2 chemokines. The activated platelets may then further act on the recruited neutrophils, leading to release of NETs to more effectively protect the body from foreign pathogens.

Role of platelets in acquired immunity

Platelets are known to also play a role in antigen-specific acquired immunity. For instance, platelets from allergic donors expressed both high- and low-affinity IgE receptors on their surface, and exposure to appropriate antigens led to production of inflammatory mediators such as serotonin and CCL5 (also known as "regulated on activation, normal T cell expressed and secreted"; RANTES).[56–59] In a murine model of OVA-induced allergic inflammation, platelets from OVA-sensitized wild-type mice, but not mice lacking high-affinity IgE receptor, migrated to the lung in response to allergen-specific stimuli, suggesting that platelets may actively contribute to antigen-dependent allergic inflammation.[60]

Recently, Hayashizaki *et al.* reported that platelets are necessary for recruitment of antigen-specific CD69-expressing CD4 T cells into inflamed lung tissue.[61] In antigen-specific airway inflammation, platelets adhered to the vascular endothelial cell surface of the lung, and myosin light-chain 9/12 (Myl9/12) contained in the platelets was released to form intravascular net-like structures. Myl9/12 bound to its ligand, CD69-expressing CD4 T cells, thereby allowing these antigen-specific helper T cells to invade extravascular, inflammed lung tissues. Furthermore, blockade of CD69-Myl9/12 interaction effectively reduced airway eosinophilic inflammation in an antigen-induced murine model of bronchial asthma, suggesting that platelets are critically involved in antigen-specific T–cell responses.

Role of platelets in the pathogenesis of bronchial asthma

Bronchial asthma is a chronic inflammatory disease of the airways that is immunologically characterized by type 2 inflammation with increased eosinophilic infiltration of the airway.[62] In asthmatic airway, persistent structural changes in the airway walls due to chronic airway inflammation, called "airway remodeling", are known to exacerbate the clinical condition.[63] Airway remodeling includes goblet cell hyperplasia, shedding of epithelial cells, thickening of the basement membrane, extracellular matrix deposition and fibrosis, smooth muscle cell hypertrophy/hyperplasia and accelerated submucosal angiogenesis.[64–67]

To date, a number of studies investigated the association between bronchial asthma and platelets. For instance, platelets of patients with bronchial asthma are known to be more activated than those of healthy individuals.[68–72] In house-dust-sensitive patients, plasma levels of β-thromboglobulin, PF-4 and soluble P-selectin were increased after loading of mite antigen.[73] Activated platelets were able to produce platelet-derived microparticles (PMP), and Duarte *et al.* proposed that increased circulating PMP might serve as a biomarker for bronchial asthma.[74] Furthermore, platelets were shown to be actively involved in the processes of asthma progression, such as eosinophilic infiltration into the airway walls and airway remodeling. For instance, in a murine model of asthma using ovalbumin (OVA) antigen, platelet-depletion effectively ameliorated both OVA-induced infiltration of various leukocytes, including eosinophils, into the lung and airway remodeling.[75–77]

On the other hand, although substantial platelet activation is observed in patients with allergic diseases, including bronchial asthma, it should be noted that these patients tend to have a mild hemostatic defect, rather than increased incidence of thrombosis.[78,79] This may explain why platelets activated due to continuous inflammatory stimuli release various mediators, and are thus refractory to further activation (aggregation). Such platelets are thought to represent an "exhausted platelet" phenotype resulting from having been continuously activated *in vivo*.[80] A recent randomized, double-blind, placebo-controlled crossover study showed that prasugrel, an anti-platelet drug that targets the platelet P2Y12 receptor, slightly improved airway hyperresponsiveness measured by the mannitol test, while the difference between the prasugrel-treated and placebo-control groups was borderline statistically significant.[81] Meanwhile, prasugrel treatment did not change the fractional exhaled NO (FeNO) level, a surrogate marker for eosinophilic airway lung inflammation.[81] Therefore, further study is needed as to whether "exhausted platelets" can be a true target for asthma treatment.

Aspirin-exacerbated respiratory disease (AERD), which has three major symptoms, i.e., asthma, eosinophilic sinusitis and aspirin intolerance, is considered to be a special type of bronchial asthma. In AERD, asthma-like symptoms are thought to be caused by reduced production of prostaglandin E2 and excessive production of cysteinyl leukotriene (cysLT) as a result of aspirin's inhibition of cyclooxygenase-1 activity.[82,83] Indeed, in AERD patients, platelet activation was observed even at times of no attack, and activated platelets and eosinophils formed complexes.[84,85] Although platelets themselves cannot produce cysLT, eosinophil-adherent platelets can convert the leukocyte-derived precursor leukotriene (LT) A4 to LTC4 in a transcellular manner.[83] Thus, platelet activation can be observed in various types of bronchial asthma and may be involved in their pathogenesis.

Why are platelets activated in allergic inflammation and bronchial asthma?

Next, we will discuss the causes and immunological implications of platelet activation in patients with bronchial asthma. In the asthmatic airway, microvascular abnormalities such as accelerated angiogenesis and increased vascular permeability are commonly observed, and the degree of the abnormalities is known to be significantly associated with disease severity and decreased respiratory function.[64–67] It is presumed that blood vessels in the asthmatic airway tend to be damaged by various inflammatory cells and mediators. Therefore, it may be that, in order to repair such blood vessels, platelets may be always activated in asthmatics as compared with healthy individuals. Since platelets constitutively contain active IL-33 protein,[52] we hypothesize that activated platelets maintain levels of functional IL-33 in the airway, as a result of which its target cells, including ILC2, are mildly but continuously activated. In patients with bronchial asthma, chronic type 2 inflammation mediated by active platelet-derived IL-33 may serve as the basis for development and progression of the disease. In a murine model of papain-induced eosinophilic inflammation in the airway, heat-inactivated papain did not induce airway eosinophilia at all,[49,52] indicating that the protease activity of papain was necessary for induction of airway inflammation in that model. Such strong tissue injury activity by the protease may reach the microvessels beyond the airway epithelium in that model, leading to airway eosinophilia through promotion of platelet-derived IL-33 activation. Viral infection in the airways also causes tissue injury, leading to activation of alarmin IL-33. Figure 2 depicts the interactions among the cells/cytokines involved in the type 2 innate immune system, centering on ILC2 in the asthmatic airways, based on reports to date. According to this, IL-33 is certainly likely to play a central role in orchestrating communications among all cells involved in type 2 innate immune responses, including tissue cells and immune cells.

Role of IL-33 in the immune defense system

Both platelets and IL-33 are activated by tissue damage and act as crucial initiators of tissue repair. In particular, platelets are essential components for rapid hemostasis when blood vessels are

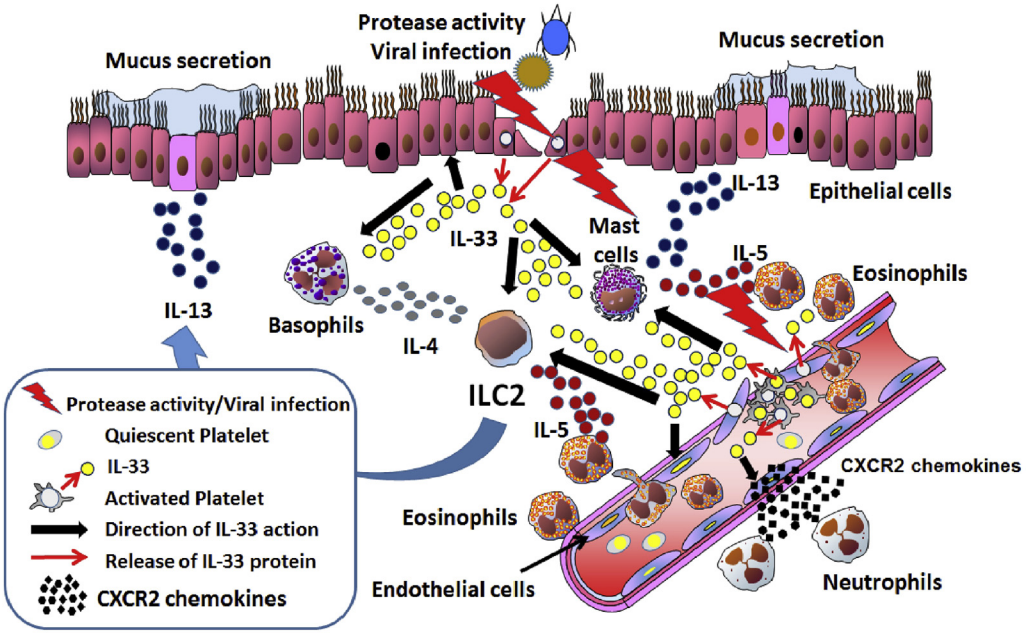

Fig. 2. Type 2 inflammatory responses to airway tissue injury. Viral infection- and/or protease antigen-induced airway tissue injury causes IL-33 protein to be released from the damaged tissue. When tissue damage extends to microvessels beyond the airway epithelium, activated platelets initiate repair of the injured vessels. Also, platelet-derived IL-33 is activated in concert with tissue-derived alarmin IL-33, and they target cells such as ILC2, leading to type 2 inflammation in the airway. In particular, when pathological angiogenesis is enhanced and fragile new blood vessels are increasing, platelet activation may be further enhanced in order to repair those vessels. Platelet-derived IL-33 activity is also assumed to be maintained at a high level.

damaged and bleeding. Although IL-33 is an essential cytokine for type 2 inflammation in the airways, the action of IL-33 on tissue cells (including vascular endothelial cells) centers on migration and activation of neutrophils ([50]; and Matsuda *et al.*, unpublished data). An important advantage of IL-33 protein's presence in platelets as a biological defense mechanism is that it can act on intact tissue cells near injured blood vessels to produce large amounts of CXCR2 chemokines at the same time that hemostasis is occurring. This would be a reasonable system as the frontline of biological defense since the chemokines would rapidly recruit neutrophils to lesion sites (Fig. 1B). Activated platelets may then further act on the accumulated neutrophils, leading to release of NETs to more effectively protect the body from foreign pathogens. In addition, IL-33 was found to possess anti-fungal activity through CXCR2 chemokine, which acts directly on neutrophils and elevates their migration and phagocytosis activities at sites of infection.[86] In a murine experimental sepsis model induced by cecal ligation and puncture, IL-33 effectively reduced mortality by recruiting neutrophils to the site of inflammation.[87] These results also show IL-33's participation in rapid immune defense via neutrophils and bacterial clearance, suggesting multiple roles for IL-33 in immune defense.

Conclusion

Taken together, platelets and IL-33 may play at least in part a common role *in vivo*. That is, both platelets and IL-33 respond to tissue damage and are promptly involved in biological defense mechanisms and initiation of the tissue repair process. From this perspective, activated platelets may also be regarded as an alarmin that convey a danger signal. Although platelets are anuclear cells derived from the cytoplasm of megakaryocytes, in emergency situations such as trauma and infection, activation of a defense system as quickly as possible is essential. For that reason, since a control mechanism routed through de novo gene expression in cell nuclei would likely be too cumbersome and slow to respond to an urgent threat, platelets, which already contain various mediators, seem well–suited for dealing with emergency situations. On the other hand, while inhaled corticosteroids are used as a standard treatment for bronchial asthma, they will not target or affect platelets. That is because the anti-inflammatory action of corticosteroids is mediated via nuclear glucocorticoid receptors, but platelets have no nucleus. Therefore, platelets may be involved in the development of steroid–refractory asthma, including irreversible airway remodeling, but further study of this point is needed.

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Conflict of interest

The authors have no conflicts of interest to declare.

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