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Review article

Recent advances in understanding the roles of vascular endothelial cells in allergic inflammation

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AD, atopic dermatitis; BA, bronchial asthma; CCR, CC chemokine receptor; COPD, chronic obstructive pulmonary disease; GWAS, genome-wide association studies; ICAM, intercellular adhesion molecule; ICS, inhaled corticosteroid; ILC, innate lymphoid cells; MHC, major histocompatibility complex; PAMPs, pathogen-associated molecular patterns; PMCH, pro-melanin-concentrating hormone; PRRs, pattern-recognition receptors; TARC, thymus and activation-regulated chemokine; TSLP, thymic stromal lymphopoietin; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor

ABSTRACT

Allergic disorders commonly involve both chronic tissue inflammation and remodeling caused by immunological reactions to various antigens on tissue surfaces. Due to their anatomical location, vascular endothelial cells are the final responders to interact with various exogenous factors that come into contact with the epithelial surface, such as pathogen-associated molecular patterns (PAMPs) and antigens. Recent studies have shed light on the important roles of endothelial cells in the development and exacerbation of allergic disorders. For instance, endothelial cells have the greatest potential to produce several key molecules that are deeply involved in allergic inflammation, such as periostin and thymus and activation-regulated chemokine (TARC/CCL17). Additionally, endothelial cells were recently shown to be important functional targets for IL-33—an essential regulator of allergic inflammation. Notably, almost all endothelial cell responses and functions involved in allergic inflammation are not suppressed by corticosteroids. These corticosteroid-refractory endothelial cell responses and functions include TNF-α-associated angiogenesis, leukocyte adhesion, IL-33-mediated responses and periostin and TARC production. Therefore, these unique responses and functions of endothelial cells may be critically involved in the pathogenesis of various allergic disorders, especially their refractory processes. Here, we review recent studies, including ours, which have elucidated previously unknown pathophysiological roles of vascular endothelial cells in allergic inflammation and discuss the possibility of endothelium-targeted therapy for allergic disorders.

Introduction

The global prevalence of allergic disorders, including a range of chronic illnesses, such as bronchial asthma (BA),[1] allergic rhinitis,[2] eosinophilic gastrointestinal disorders[3] and atopic dermatitis (AD),[4] has been increasing in recent decades, leading to serious social and economic burdens.[5] The pathogenesis of these allergic disorders commonly involves both chronic tissue inflammation and remodeling.[6]

The pathogenesis of allergic diseases is characterized by chronically progressive inflammatory reactions that are often triggered by exposure of epithelial surfaces to antigens. Epithelial inflammation leads to responses by tissue structural cells, in addition to activation of a variety of immune cells such as lymphocytes, phagocytes and granulocytes, to the antigens that play essential roles in the pathogenesis of the inflammation. Among

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tissue structural cells, epithelial cells constitute the initial structural and immunological barrier to entry of foreign antigens. In addition, recent studies strongly suggest that epithelial cells also function as important sources of a wide variety of immune mediators. In particular, novel epithelial cell-derived cytokines, including IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), have attracted considerable interest because of their potent functions in promoting type 2 immunity.[7] Fibroblasts, another type of tissue structural cell, play crucial roles in maintaining tissue homeostasis and bringing about wound healing under normal conditions. However, excessive and chronic inflammation as a consequence of activation of immune responses often leads to irreversible tissue fibrosis, resulting in severe and refractory illnesses.[8] Compared to epithelial cells and fibroblasts, the roles of vascular endothelial cells in allergic disorders remain poorly understood. Figure 1A shows a simplified structure of the tissue surface.

Endothelial cells form a one-cell-thick layer called the endothelium (Fig. 1A), which lines the inner wall of blood vessels, forming a selectively permeable barrier between the blood inside the vessels and the surrounding tissues. The endothelium is a highly specialized structure spread throughout the body. It has many vital functions, including blood and oxygen supply, nutrient delivery, metabolic homeostasis and immune cell trafficking.[9–13] On the other hand, we have elucidated several critical roles of pulmonary and skin microvascular endothelial cells in allergic inflammation. In particular, endothelial cells have the greatest potential to produce several key molecules involved in allergic inflammation. The blood levels of periostin[14] and thymus and activation-regulated chemokine (TARC/CCL17)[15] already serve as reliable biomarkers reflecting disease progression of allergic disorders. In addition to being key cellular sources of molecules essential for allergic inflammation, endothelial cells are important functional targets for IL-33,[16] whose gene locus has been reported to be the most consistently associated with BA in all tested ethnic groups. Notably, almost all endothelial cell responses and functions involved in allergic inflammation cannot be suppressed by corticosteroid treatment, a current first-line therapy for various allergic disorders. We, therefore, inferred that endothelial cell responses and functions are crucially involved in the progression of various allergic disorders, especially in corticosteroid-refractory processes.

This review article focuses on the contributions of vascular endothelial cells in the development and exacerbation of allergic disorders. Following a brief introduction of the basic biology, we will review how recent reports, including ours, have elucidated the responses of endothelial cells to representative type 2 cytokines and chemokines in allergic airway and skin inflammation. We finally discuss the roles of endothelial cells in corticosteroid refractoriness and the possibility of endothelium-targeted therapy for allergic disorders.

Structure and fundamental functions of endothelial cells

Blood vessels are composed of a sheet of inner endothelium, which is a monolayer of endothelial cells that surrounds functional cells, including pericytes and vascular smooth muscle cells, and extracellular matrix (Fig. 1A).[17] The endothelial and supportive mural cells are tightly bound to each other, partly through integrins, thereby maintaining vascular integrity (Fig. 1A). The endothelium is composed of $1–6 \times 10^{13}$ endothelial cells that cover more than 1000 m^2 of surface area throughout the body.[9,10]

In the absence of inflammation, vascular endothelial cells serve as an essential barrier between the bloodstream and vessel walls. In addition to being a physical barrier, endothelial cells have various indispensable functions, which can be classified into three major groups: 1) modulation of metabolic homeostasis (trophic action), 2) control of vascular hemodynamics (tonic action) and 3) regulation of vascular permeability, coagulation and cell extravasation (trafficking).[18]

These functions of endothelial cells change during the transition from quiescent to inflammatory conditions. The initial change of the vessels in acute inflammation is characterized by increased blood flow secondary to arteriolar and capillary bed dilation (erythema and warmth).[19,20] Increased vascular permeability, as a consequence of either widening of interendothelial cell junctions of the venules or direct endothelial cell injury, results in an exudate of

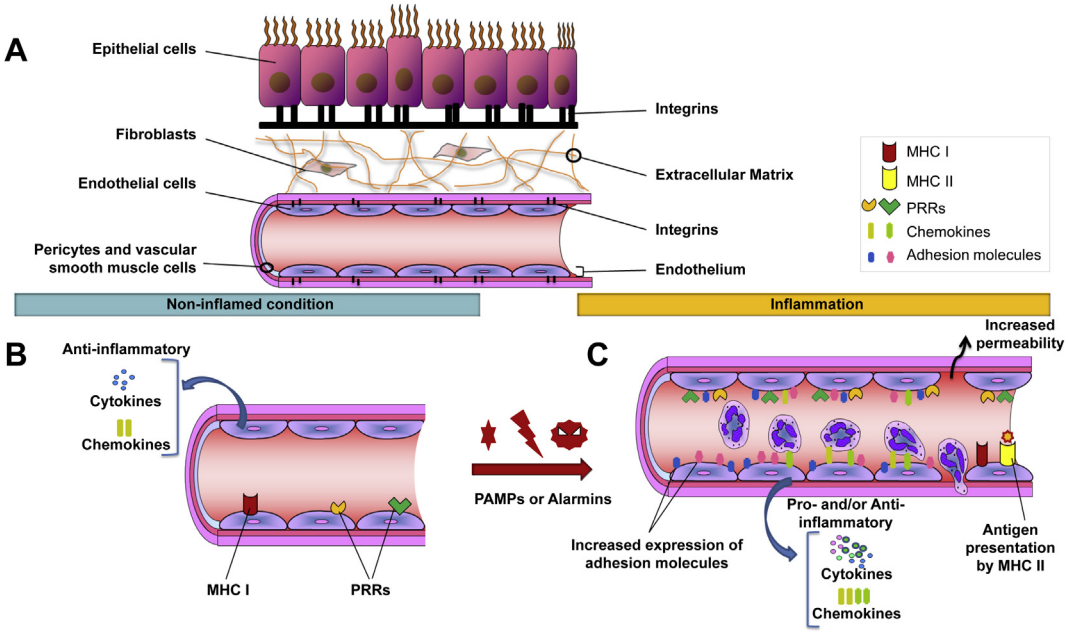

Fig. 1. The basic biology of vascular endothelial cells. (A) Simplified structure of the tissue surface. In addition to being a physical barrier, endothelial cells have several indispensable functions. The different roles of endothelial cells are illustrated in non-inflamed conditions (B) and in inflammation (C).

protein-rich extravascular fluid (tissue edema).[21,22] Endothelial cells normally maintain a balance between the pro- and anti-coagulant activities, but they express numerous pro-coagulant factors in response to injury or inflammatory cytokines. Endothelial injury exposes the underlying subendothelial von Willebrand factor and basement membrane collagen, stimulating platelet adhesion, platelet activation and aggregation. These interactions of platelets and endothelium have a profound impact on clot formation as a defense mechanism. Concurrently, leukocyte recruitment from the bloodstream into the extravascular tissue at sites of pathogen invasion or tissue damage is a multi-step process: 1) loose attachment to and roll-over on the endothelium (mediated by selectins and carbohydrates on endothelial cells), 2) firm and selective attachment to the endothelium (mediated by integrins and chemokines), 3) migration through interendothelial spaces and 4) detachment from the blood vessel.[23–25] Thus, these structural and functional changes of endothelial cells facilitate leukocyte recruitment and alter gene expression profiles in inflammatory conditions and are collectively called “endothelial activation”.[26]

Roles of endothelial cells in allergic inflammation

The roles of endothelial cells in innate and adaptive immune responses

Allergic disorders commonly involve both chronic tissue inflammation and remodeling caused by immunological reactions to various pathogen-associated molecular patterns (PAMPs) and/or endogenous danger substances released from damaged tissues, i.e., so-called alarmins. Due to their anatomical location, vascular endothelial cells are the final responders to interact with various alarmins on the epithelial surface. Endothelial cells, as well as epithelial cells, actively participate in both innate and adaptive immune responses, which are crucially involved in the pathogenesis of allergic disorders.[27,28]

The primary inflammatory response of tissue cells is initiated by recognition of various PAMPs and alarmins via pattern-recognition receptors (PRRs); this is called innate immunity. Indeed, vascular endothelial cells spontaneously express class I major histocompatibility complex (MHC) molecules and a wide variety of functional PRRs, including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs), as reviewed by Danese *et al.*,[29] as well as various cytokine and chemokine receptors (Fig. 1B).[30,31] Endothelial cells are also capable of secreting a broad spectrum of cytokines and chemokines in response to stimulation. As a result, endothelial cells participate in the immune response and rapidly produce various inflammatory molecules (e.g., IL-1α, IL-1β, IL-6, IL-8, TNF-α, etc.)[32,33] that are important for endothelial activation (Fig. 1C). Concurrently, endothelial cells can also produce anti-inflammatory cytokines and chemokines that prevent progression of the inflammatory cascade.[34] The inflammatory mediators involved in those inflammatory cascades or released by endothelial cells were reviewed and discussed in depth in a recent review article by Mai *et al.*[28]

In addition, in response to some inflammatory stimuli, endothelial cells express class II MHC molecules that present endothelial antigens to immune cells, leading to long-lasting and highly specific protection, known as adaptive immunity (Fig. 1C).[35–37] Thus, vascular endothelial cells play pivotal roles in both innate and adaptive immune responses.

Cascade of allergic inflammation regulated by structural cells

Allergic inflammation is regulated by complex interactions among several inflammatory and structural cells via inflammatory mediators.[1] A wide variety of mediators, cytokines and chemokines exerting many different effects on the airways and skin are known to be involved in the pathology of allergic disorders, maintaining chronic allergic inflammation. A well-known cascade of these networks in allergic inflammation is characterized by a type 2 immune response with production of specific cytokines and chemokines initiated by allergen exposure.[38] In addition to acute responses, cytokines and chemokines produced by Th2 cells, mast cells and basophils recruit eosinophils from the blood into the airways and skin, leading to allergic asthma and atopic dermatitis. As noted earlier, besides hematopoietic cells, structural cells such as epithelial cells, fibroblasts and smooth muscle cells are known to contribute to allergic inflammation. Nevertheless, the roles of vascular endothelial cells in allergic inflammation had not yet been well studied.

Endothelial cells in the lung

Airway remodeling and angiogenesis

Bronchial asthma (BA) is a chronic inflammatory disease of the airways that is characterized by airway hyperresponsiveness, episodic airway obstruction and a decline in lung function. In some patients with BA, chronic pulmonary inflammation results in persistent structural changes in the airway walls, including goblet cell hyperplasia, shedding of epithelial cells, thickening of the basement membrane, extracellular matrix deposition and fibrosis, smooth muscle cell hypertrophy/hyperplasia and accelerated angiogenesis.[39] These structural changes are collectively called “airway remodeling”, which is thought to lead to irreversible airway obstruction and exacerbation of BA. Angiogenesis is a complicated, multiphase process regulated by several factors, including molecules closely related with the pathogenesis of asthma.[40–42] Previous studies have suggested that, among the above changes underlying airway remodeling, accelerated angiogenesis occurs even in the early stage of chronic BA[43] but is not seen in the airway wall in chronic obstructive pulmonary disease (COPD), another pulmonary chronic inflammatory disease.[43] Thus, angiogenesis, the growth of new vessels from existing ones, is one of the most important pathological features of BA.

Vascular endothelial growth factor (VEGF), an endothelial cell-specific mitogen essential for angiogenesis,[44] has been reported to be critically involved in angiogenesis in the asthmatic airway. For instance, VEGF is abundant in asthmatic airways compared to healthy control airways.[45–47] Notably, VEGF levels in the airways show a positive correlation with the severity of airway inflammation[48] and are associated inversely with lung function,[49] suggesting that VEGF plays important roles in BA disease progression. On the other hand, a number of reports demonstrated that inhaled corticosteroid (ICS) treatment effectively inhibited VEGF overproduction in the airways, resulting in reduced pulmonary angiogenesis and improved lung function, especially in mild asthmatic patients.[50–52] Importantly, however, some patients with severe BA often exhibit increased pulmonary angiogenesis regardless of long-term treatment with corticosteroids. These pathological findings suggest that increased pulmonary angiogenesis, especially in severe BA, may be poorly responsive to corticosteroid therapy and clinically associated with reduced lung function. Therefore, factor(s) other than VEGF may be involved in the corticosteroid-refractory angiogenesis in severe BA.

Both elevated levels of TNF-α and neutrophilic inflammation in the airway are reported to be characteristic features in some patients with severe asthma.[53–55] We previously showed that type 2 cytokines such as IL-4 or IL-13, but not type 1 cytokines such as IFN-γ, significantly promoted *in vitro* angiogenesis by human pulmonary microvascular endothelial cells, but only in the co-presence of TNF-α.[56] Importantly, the TNF-α- and type 2 cytokine-mediated

angiogenesis was provoked by autocrine stimulation of CXCR2 chemokines, including IL-8, growth-related oncogene (GRO), epithelial neutrophil-activating peptide 78 (ENA78) and granulocyte chemotactic protein 2 (GCP-2). It should be noted that the CXCR2 chemokines can act not only as potent neutrophilic chemoattractants mediated via CXCR2 receptors on neutrophils, but also as potent angiogenic factors mediated via CXCR2 receptors on microvascular endothelial cells.[57,58] Thus, the increased airway angiogenesis as well as neutrophilic inflammation in corticosteroid refractory severe asthma might be primarily due to TNF-α and CXCR2 chemokines, rather than mediation by VEGF. In fact, both IL-8 and TNF-α are reported to be abundant in severe asthmatic airways.[53,54,59,60]

Endothelial cells and IL-33

Complex interactions between environmental factors and genetic variants are at the heart of the pathogenesis of BA. To date, a number of studies investigated the genetics of BA. Several recent large-scale genome-wide association studies (GWAS) found that both *IL33* and its receptor, *IL1RL1/ST2*—which are located on different chromosomes—are the most consistently associated genes for asthma, regardless of race differences.[61–63] IL-33, a member of the IL-1 family of cytokines, is a tissue-derived factor and may be involved in type 2 immunity. Although IL-33 was originally identified as nuclear factor from high-endothelial venules (NF-HEV),[64] subsequent studies revealed that it is constitutively expressed in the nucleus of such tissue cells as epithelial and endothelial cells and is released as an alarmin by damaged tissue cells after injury and/or trauma.[65] The target cells of IL-33 express IL-33 receptors[66] and include various effector cells such as group 2 innate lymphoid cells (ILC2),[67,68] Th2 cells,[69] eosinophils,[70] basophils,[71,72] dendritic cells[73] and mast cells.[74] These effector cells abundantly produce type 2 cytokines such as IL-5 and IL-13 upon IL-33 stimulation. In addition, upon exposure to IL-33, dendritic cells are activated and facilitate naïve T cell development towards atypical Th2 cells which produce IL-5 and IL-13 but almost no IL-4.

We reported that IL-33 can stimulate pulmonary microvascular endothelial and epithelial cells via the IL1RL1/ST2 receptor, and those cells then immediately and strongly produce CXCR2 chemokines, including IL-8.[16] Those findings suggest that such tissue structural cells as epithelial and endothelial cells play roles not only as significant sources of IL-33, but also as important functional targets of IL-33. IL-13 as well as IL-4 significantly enhanced IL1RL1/ST2 expression and function in both epithelial and endothelial cells in a signal transducer and activator of transcription 6 (STAT6)-dependent manner.[16] These observations suggest that allergic individuals, who have type 2-skewed immunity, might be more sensitive to IL-33-mediated inflammatory responses by tissue cells than non-allergic individuals.

As described above, CXCR2 chemokines, such as IL-8, are indispensable for pulmonary angiogenesis.[56] Since IL-33 can promote strong CXCR2 chemokine production by pulmonary endothelial and epithelial cells,[16] IL-33 might be involved in pulmonary angiogenesis. In fact, a recent report demonstrated that IL-33 promotes angiogenesis and vascular permeability by stimulating endothelial nitric oxide production via the IL1RL1/ST2 receptor.[75] Taken together, IL-33, a potent type 2-promoting cytokine, can act directly on pulmonary tissue cells and is crucially involved in airway remodeling, including angiogenesis.

Endothelial cells in the skin

The skin and allergy

The skin is the largest organ of the human body, and recent evidence has begun to highlight the critical role of the skin in the pathogenesis of multiple allergic disorders, in addition to its primary function as a structural barrier.[76] In fact, we recently reported that daily application of a moisturizer to neonates with a family history of allergic disorders during the first 32 weeks of life can significantly reduce the risk of AD/eczema.[77] These observations suggest that skin barrier function is directly involved in the development of AD. The skin is structurally composed of two different layers, the epidermis and dermis. Whereas the epidermis is an avascular layer of stratified squamous epithelium, the dermis is highly vascularized and composed largely of extracellular matrix components. The dermal vasculature is organized into a deep and a superficial horizontal plexus, and it might play a distinct role in skin allergy.

Endothelial cells in atopic dermatitis; involvement of thymus and activation-regulated chemokine (TARC/CCL17)

Atopic dermatitis (AD) is characterized by chronic or relapsing eczematous lesions and recurrent pruritus.[78] AD lesions are critically associated with activated vascular endothelial cells, and the cutaneous vasculature thus plays key roles in various clinical symptoms of AD, including erythema, edema, effector cell recruitment and white dermographism. Various effector cells, including lymphocytes, phagocytes and granulocytes, communicate closely with the endothelium and activate each other via paracrine stimulation of inflammatory mediators. Furthermore, adhesion, tethering and transmigration of infiltrating effector cells are also highly regulated, active communication processes between endothelial cells and those effector cells. Thus, specific interactions between activated endothelial and effector cells are essentially involved in the establishment and progression of AD pathology.

Thymus and activation-regulated chemokine (TARC/CCL17), a member of the CC chemokine family, is a potent and selective chemoattractant for Th2 cells via CC chemokine receptor 4 (CCR4).[79] There is increasing evidence that TARC is involved in the development of various allergic disorders, and elevated TARC blood levels were especially seen in AD patients.[80,81] Importantly, serum TARC levels already serve as a reliable biomarker reflecting disease progression of AD in daily clinical practice, especially in Japan.[82] However, the reason that serum TARC levels correlate well with the degree of AD progression remains unclear. Although physicians traditionally check the condition of skin to determine whether a particular treatment has been successful or not, visual examination results may not always be accurate and reliable. This inaccuracy might be due to the existence of subclinical dermal inflammation, which is often associated with severe and corticosteroid-refractory AD.[15] In fact, it is well known that AD patients with elevated serum TARC levels tend to relapse easily and fail to achieve good control of AD symptoms, even when their skin lesions seem to disappear after initial anti-inflammatory therapy.

These observations indicate that serum TARC levels in AD patients correlate well with the degree of subclinical inflammation in the skin. Therefore, we hypothesized that dermal cells may be major cellular sources of TARC in AD patients and may play crucial roles in subclinical inflammation in the AD skin. Indeed, we recently demonstrated that both dermal microvascular endothelial cells and fibroblasts, but not epidermal keratinocytes, possess vast potential to produce TARC protein in response to TNF-α plus IL-4 or IL-13.[83] Anti-TARC mAb reacted specifically with microvascular endothelial cells of the dermis, but not with epidermal cells, at sites of $CCR4^{+}$-T cell accumulation in inflamed AD skin,[84,85] further suggesting that TARC derived from endothelial cells may be functionally associated with the pathophysiology of AD.[86] Taken together, complete inhibition of inflammation in the dermis is thought to be particularly important for suppressing not only the TARC blood level, but also progression of AD, even if epidermal eczematous lesions seem to be improved.

Essential roles of endothelial cells in corticosteroid refractoriness of allergic disorders

Corticosteroids are currently the most effective anti-inflammatory agents for treating allergic disorders, including BA[87] and AD.[82] However, the therapeutic response to corticosteroids varies among individuals and with the disease subphenotype, and the mechanisms underlying corticosteroid refractoriness remain obscure. We examined the effectiveness of corticosteroids on various tissue cells involved in allergic disorders and found cell- and stimulant-dependent effects. In particular, the impact of corticosteroids on microvascular endothelial cells is apparently distinct from that on other airway and skin structural cells, including epithelial cells, smooth muscle cells and fibroblasts.[88] We therefore surmise that microvascular inflammation in allergic disorders may be very important to corticosteroid refractoriness.

Corticosteroid-refractory angiogenesis

As described in an earlier section (*Airway remodeling and angiogenesis*), VEGF-dependent pulmonary angiogenesis, especially in mild BA patients or *in vitro* experiments, tends to be highly responsive to corticosteroid therapy, whereas severe BA patients often exhibit increased pulmonary angiogenesis in spite of long-term corticosteroid treatment. We demonstrated that, only in the presence of TNF-α, type 2 cytokines such as IL-4 and IL-13 significantly promoted *in vitro* pulmonary angiogenesis through autocrine stimulation of CXCR2 chemokines, including IL-8.[56] Importantly, CXCR2-regulated angiogenesis was not inhibited by corticosteroid treatment, because corticosteroids have little effect on TNF-α-induced CXCR2 chemokine production by human pulmonary microvascular endothelial cells.[88,89] These observations further support the notion that TNF-α/CXCR2 chemokine-regulated corticosteroid-refractory angiogenesis might be crucially involved in the progression of severe BA.

Corticosteroid effects on leukocyte adhesion to endothelial cells

Although such phenotypic changes in severe asthma as increased TNF-α and neutrophilic inflammation are thought to contribute to corticosteroid refractoriness,[90] the underlying mechanisms of acquisition of corticosteroid refractoriness are not fully understood. Once tissue cells recognize PAMPs or alarmins, multiple pro-inflammatory molecules that are necessary for leukocyte recruitment into inflamed tissues act on the vascular endothelium. This recruitment is an important step in the development of acute and chronic inflammatory responses[91] and is a dynamic process requiring mutual interactions between leukocytes and the activated vascular endothelium. TNF-α is the most prominent factor in vascular endothelium activation. To that end, TNF-α can induce expression of various molecules on the vascular endothelium that are essential for leukocyte recruitment into inflamed tissues, including intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1).[92]

We demonstrated that TNF-α-induced expression of ICAM-1 and VCAM-1 on human pulmonary microvascular endothelial cells was further enhanced by corticosteroid treatment, via its receptors.[89] The corticosteroid treatment also enhanced *in vitro* adhesion of neutrophils and eosinophils to the TNF-α-treated endothelial cells.[89] These observations may explain, at least in part, corticosteroid refractoriness that is seen especially in severe asthma, which is characterized by markedly elevated TNF-α production. In agreement with our findings, the total number of vessels expressing ICAM-1 was significantly higher in severe corticosteroid-dependent asthmatic airways than in mild asthmatics and control subjects.[93] In contrast, TNF-α-induced ICAM-1 expression by airway epithelial and smooth muscle cells was effectively inhibited by corticosteroid treatment (our unpublished data). Therefore, the side effects of corticosteroid might be seen as specific features of microvessels in the airway, but the underlying molecular mechanism for such *in vitro* observations still needs to be elucidated.

Corticosteroid actions on IL-33-mediated responses of endothelial and epithelial cells

As described in an earlier section (*Endothelial cells and IL-33*), IL-33, a potent type 2-promoting cytokine, can induce production of CXCR2 chemokines (such as IL-8) that are major neutrophil chemoattractants as well as pro-angiogenic factors, by both pulmonary microvascular and epithelial cells. We also found that IL-33-induced IL-8 production by epithelial cells was almost completely abolished by corticosteroid treatment. In contrast, that same response in microvascular endothelial cells was only partially abolished by corticosteroid.[16] These findings further suggest that the poor effect of corticosteroids on TNF-α- and IL-33-mediated inflammatory responses of microvascular endothelial cells, including corticosteroid-refractory angiogenesis, might be crucially involved in the refractoriness seen in asthmatics.

Cell type-dependent effects of corticosteroid on periostin production

Periostin is a matricellular protein originally isolated from an osteoblast cell line.[94] This molecule has recently received much attention in the field of allergy research because of its pivotal role in the chronicity of allergic inflammation as a component of corticosteroid-refractory tissue fibrosis.[14,95,96] Furthermore, serum periostin levels serve as a systemic biomarker reflecting development of airflow limitation[97] and airway eosinophilic inflammation,[98] even in asthmatic patients receiving high doses of corticosteroid treatment. Therefore, we hypothesized that there must be an important cellular source of periostin in a corticosteroid-refractory manner. In agreement with other studies, we found that IL-4, IL-13 and TGF-β each induced considerable amounts of periostin protein production by fibroblasts derived from both lung and skin.[99] Of note, corticosteroid treatment completely inhibited IL-4/IL-13-induced, but did not affect TGF-β-induced, periostin production by fibroblasts. Microvascular endothelial cells from lung and skin showed similar periostin induction profiles as fibroblasts when treated with IL-4/IL-13 but not with TGF-β. Remarkably, corticosteroid treatment of microvascular endothelial cells further enhanced, rather than reduced, their IL-4/IL-13-induced periostin production.[99] These observations suggest that microvascular endothelial cells, along with fibroblasts, are likely to be significant cellular sources of periostin. Overproduction of periostin despite adequate corticosteroid therapy might be due to corticosteroid refractoriness to periostin production by microvessels, leading to chronic corticosteroid-refractory tissue fibrosis in the lung and/or skin. Like periostin, TARC production by microvascular endothelial cells derived from skin was further enhanced by corticosteroid treatment.[83] Interestingly, unlike periostin, TARC production by skin fibroblasts was synergistically enhanced by corticosteroid treatment. Clarification of the molecular mechanisms underlying such cell-type-dependent and molecular-specific effects of corticosteroids may help us better understand the refractory processes in allergic disorders and aid in the development of specific therapeutic strategies for severe corticosteroid-refractory allergic disorders.

Endothelial cells as possible therapeutic targets for corticosteroid-refractory allergic disorders

The unique and various effects of corticosteroids on endothelial cells, compared with on other tissue structural cells such as epithelial cells and fibroblasts, may be critically involved in refractory allergic disorders. Likewise, these observations also suggest that endothelial-targeted therapies are likely to become important treatment options for corticosteroid-refractory allergies. For instance, CXCR2 seems to be a potential therapeutic target for attenuating corticosteroid-refractory angiogenesis in the lung, as well as attenuating CXCR2 chemokine-induced neutrophil recruitment and activation, especially in patients with severe asthma, characterized by elevated levels of TNF-α and neutrophilic inflammation.[55] ICAM-1, an adhesion molecule for neutrophils, on microvessels is also a potential target for preventing neutrophil recruitment in local tissues, because ICAM-1-expressing vessels were reported to be increased in the severe corticosteroid-dependent asthmatic airway.[93] IL-33 and its receptor, IL1RL1/ST2, which are the genes most consistently associated with BA onset, can act on various potent effector cells, including ILC2 and microvascular endothelial cells. Thus, targeting the IL-33-IL1RL1/ST2 axis may be effective not only for curing BA symptoms but also for preventing the onset of BA.

Unique genes in endothelial cells

As described in an earlier section (*Airway remodeling and angiogenesis*), TNF-α and IL-4 synergistically promoted corticosteroid-refractory angiogenesis of pulmonary microvascular endothelial cells through autocrine stimulation of CXCR2 chemokines.[56] These results suggest that the responses of endothelial cells to combined stimulation with TNF-α and IL-4 might be crucial for exacerbation of BA. We therefore performed comprehensive gene expression profiling of microvascular endothelial cells in response to TNF-α plus IL-4 stimulation in order to explore new pathways and/or factors involved in the development of more severe BA. We unexpectedly found that a gene unfamiliar to us, pro-melanin-concentrating hormone (PMCH), which encodes an appetite-stimulating peptide, was by far the most strongly induced gene (over 1200-fold increase), in a STAT6-dependent manner.[100] PMCH was inhibited by corticosteroid treatment (our unpublished data) even though PMCH is an endothelial cell-specific transcript (Fig. 2 left panel). These results indicate that responses of endothelial cells can potentially be down-regulated by corticosteroids.

Melanin-concentrating hormone (MCH) is a cyclic, 19-amino-acid polypeptide that was originally isolated from the pituitary of teleost fish and found to lighten skin color by regulating melanosome aggregation.[101] In mammals, the MCH system appears to be essential for regulation of feeding behavior, energy homeostasis and anxiety-related responses.[102,103] Indeed, mice lacking the PMCH gene had a lean phenotype as a consequence of decreased appetite and increased metabolic rate.[104] Furthermore, MCH receptor-knockout mice showed reduced anxiety-like behavior[105] as well as a lean phenotype and resistance to diet-induced obesity.[106]

On the other hand, the major sources and physiological roles of MCH in the periphery remain unknown. We showed that human vascular endothelial cells from different parts of the body commonly produce considerable amounts of MCH peptide in response to such type 2 cytokines as IL-4 and IL-13, irrespective of their origin, and the production is transcriptionally regulated via STAT6.[100] Importantly, Sandig *et al.*[107] demonstrated that human Th2 cells, but not Th1 cells, can also selectively produce MCH, suggesting that type 2 inflammation might be associated with obesity and/or depression. Of note, epidemiologic studies found that allergy correlated positively with obesity[108] and depression.[109] Allergic disorders, obesity and depression, all of which are

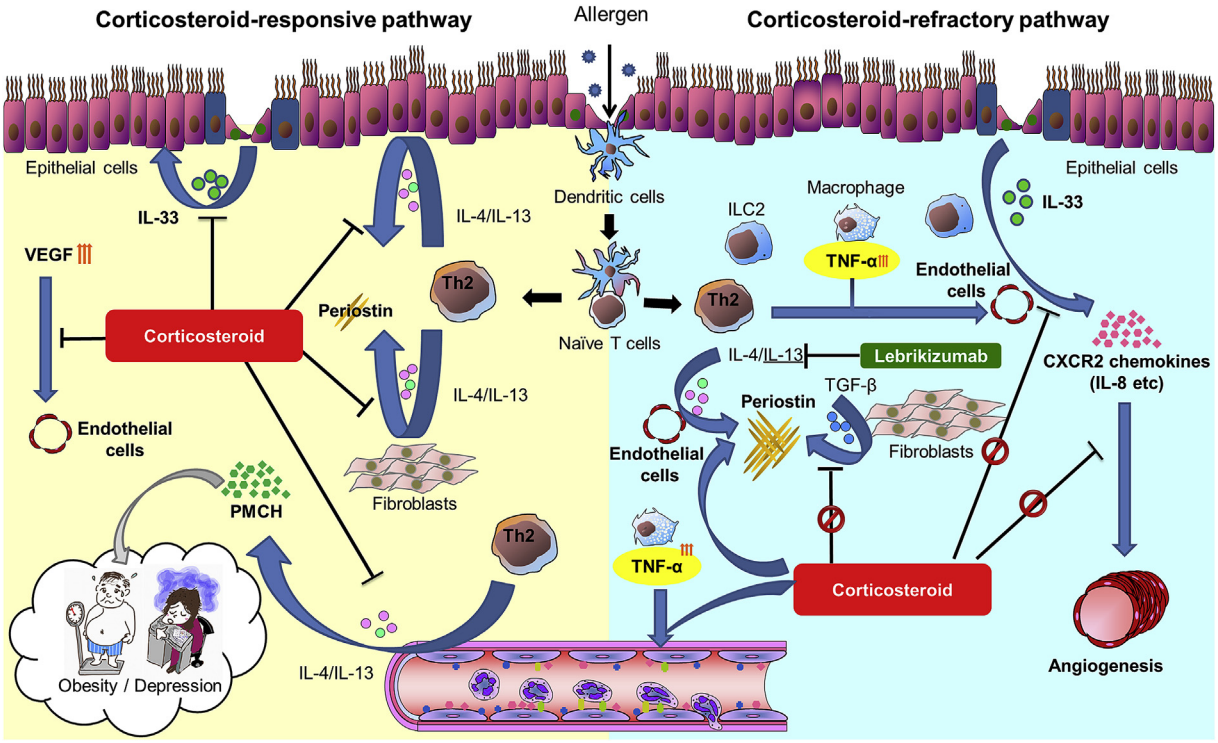

Fig. 2. The possible roles of endothelial cells in allergic airway inflammation. Schematic summary of the possible roles of endothelial cells in allergic airway inflammation, as described in this review article.

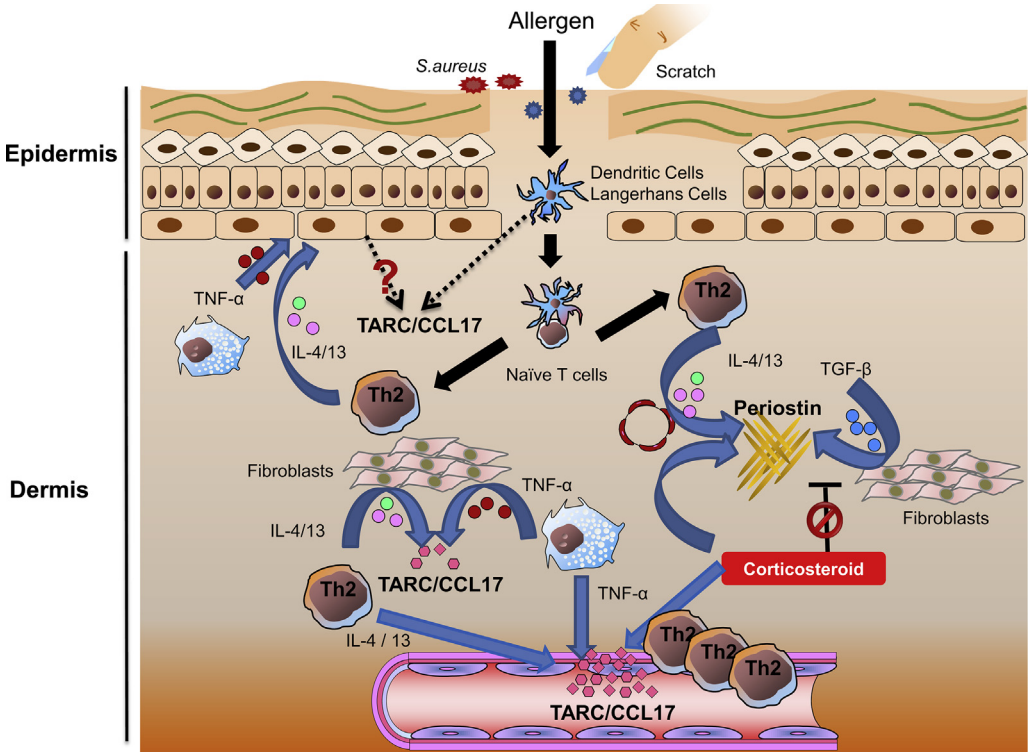

Fig. 3. The possible roles of endothelial cells in allergic skin inflammation. Schematic summary of the possible roles of endothelial cells in allergic skin inflammation, as described in this review article.

representative modern diseases, are considered to be very important worldwide public health issues. We anticipate future studies that will explore our hypothesis that MCH plays a central role in the mechanistic link between allergic inflammation and obesity or depression.

Conclusions

Recent studies have shed light on the importance of endothelial cells in the development and exacerbation of allergic disorders. Figure 2, 3 present schematic summaries of the known and putative roles of endothelial cells that have been described in this review article. These cells produce several key molecules involved in allergic disorders, such as periostin and TARC, which are not only critical to the pathogenesis of the disorders but also already serve as reliable biomarkers reflecting disease progression. Furthermore, among tissue structural cells, endothelial cells are the most significant functional targets for IL-33, which is a key regulator of allergic inflammation. Most importantly, almost all endothelial cell responses and functions relevant to allergic inflammation are not inhibited by corticosteroid treatment, a current first-line therapy for various allergic disorders. Corticosteroid-refractory endothelial cell responses and functions include TNF-α-associated angiogenesis, leukocyte adhesion, IL-33-mediated responses and periostin and TARC production. Therefore, these unique and varied corticosteroid-refractory responses and functions seem likely to be critically involved in the refractory processes of allergic disorders, and endothelial-targeted therapies may become important treatment options for corticosteroid-refractory allergic disorders. Moreover, a complete mechanistic understanding of how endothelial cells contribute to chronicity of allergic inflammation promises to provide new insights into the pathogenesis of severe allergic disorders as well as novel diagnostic and therapeutic approaches. Interestingly, in response to TNF-α plus IL-4 stimulation, the endothelial cell transcriptome showed marked upregulation of PMCH, which plays central roles in regulating feeding behavior, energy homeostasis and anxiety-related responses. MCH may be a key molecule linking allergy to obesity and depression, correlations that have already been shown epidemiologically. Thus, endothelial cell biology is an exciting and potentially high-reward research field in regard to allergies.

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Conflicts of interest

The authors have no conflict of interest to declare.

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