L4. Eosinophils: How they contribute to endothelial damage and dysfunction

Introduction

Eosinophils are poorly represented in the peripheral blood of healthy subjects (<0.5 G/L), and are found at specific sites, namely the thymus, gastrointestinal tract, mammary glands, and endometrium. Increased blood eosinophilia is found in various diseases (box 1), and is defined as hypereosinophilia when blood counts exceed 1.5 G/L. Patients with persistent hypereosinophilia often also have extravascular eosinophilic infiltrates, and may eventually experience eosinophil-mediated damage in one or more tissue(s) and organ(s). The term “hypereosinophilic syndromes” (HES) applies to all conditions wherein development of organ damage is directly related to eosinophils, whatever the underlying cause of hypereosinophilia [1].

The heart is a critical target-organ in presence of persistent hypereosinophilia, and numerous denominations have been used including Löffler’s endocarditis, endomyocardial fibrosis, and Davies’ (tropical) endomyocardial fibrosis [2]. Early studies also highlighted the frequent occurrence of arterial and venous thrombotic events in patients with HES [3], and provided histological evidence for direct eosinophil involvement in the development of vascular lesions. Increased awareness about these serious and potentially fatal consequences of uncontrolled eosinophilia, and the ensuing adoption of more aggressive treatment strategies directed towards prompt reduction of blood eosinophil counts, have resulted in decreased incidence of these complications, with a parallel improvement in survival rates [2].

The cardiovascular lesions that complicate the course of various disease conditions associated with hypereosinophilia (e.g. parasitic infections, Churg-Strauss syndrome, HES) may be histologically indistinguishable [2]. This observation led early investigators to speculate that eosinophils themselves (and not factors related to underlying disease) were the culprits, and fuelled in vitro studies exploring the effects of eosinophils and eosinophil-derived products on endothelial cells and on coagulation pathways. Overall, these studies point towards a combination of eosinophil-mediated cytotoxicity towards endothelial cells, and a pro-coagulant state favored by hypereosinophilia.

Eosinophil-mediated cytotoxicity towards endothelial cells

Because endothelial cells lining the cardiac cavities and those lining blood vessels are essentially the same, similar mechanisms account for their damage and dysfunction. The following
mechanisms of endothelial cell injury therefore apply both to the heart and peripheral vasculature.

Activated eosinophils have been shown to display direct cytotoxicity towards endothelial cells in vitro. Incubation of intact capillaries with muscle with eosinophils from hypereosinophilic patients, but not from healthy controls, resulted in significant damage to endothelial cells, with numerous holes in the membrane, cell retractions with increased intercellular spaces, and exposure of basement membranes [4]. Similar alterations were observed with supernatants of eosinophils isolated from patients. The authors hypothesized that these cytotoxic effects were mediated by eosinophil-derived cationic proteins.

Eosinophils are indeed known to contain large amounts of highly cationic proteins, respectively named eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil-derived neurotoxin (EDN), and eosinophil peroxidase (EPO) [5]. Upon eosinophil activation, these substances are released in the microenvironment, and it has been shown that significant amounts are also detectable in serum [6]. Dense deposition of cationic proteins on endocardial and small vessel endothelial surfaces has been demonstrated by immunohistochemistry on biopsies from patients with eosinophilic endomyocarditis [7–9]. These proteins have been shown to inflict damage to mammalian cells in experimental models. Eosinophil cationic protein was shown to induce formation of large non-ion-selective transmembrane channels in target cells (endothelial cells were not investigated) in one study [10]. Another group demonstrated that purified guinea pig MBP was able to inhibit growth of porcine aortic endothelial cells in vitro, and to destroy these cells completely when they had previously been cultured to confluence [11]. The cytotoxic concentrations of MBP used in vitro were comparable to the concentrations found in vivo in serum and sputum from patients with hypereosinophilic syndromes and asthma.

In addition to direct cytotoxic effects of cationic proteins, eosinophils may lyse endothelial cells through local generation of specific oxydants by EPO. Indeed, in presence of H2O2, EPO oxidizes bromide to hypobromous acid (HOBr), especially when it is attached to the cell surface, resulting in potent bromide-dependent endothelial cell cytotoxicity [7]. The authors speculated that endothelial cytotoxicity is most prominent in the heart in subjects with hypereosinophilia, because metabolically active cardiomyocytes release H2O2, fuelling local production of cytotoxic HOBr, whereas endothelial cells lining peripheral vessels are less exposed to H2O2.

**Impact of eosinophils on coagulation**

By damaging endothelial cells, eosinophils expose the underlying tissue, naturally triggering the extrinsic coagulation pathway. However, several studies have shown that eosinophils also directly affect various factors implicated in coagulation, further enhancing the proclivity towards thrombus formation in patients with hypereosinophilia.

Eosinophil-derived MBP, ECP, and EPO can bind electrostatically to and inactivate endothelial cell-expressed thrombomodulin, thereby interfering with its anticoagulant properties [8,12]. Indeed, thrombomodulin is a highly anionic molecule that normally binds thrombin, and favors proteolytic activation of protein C rather than cleavage of fibrinogen. Eosinophil peroxidase has been shown to preferentially generate hypothiocyanous acid (HOSCN) in presence of H2O2 and its three
potential substrates bromide, nitrite, and thiocyanate [13]. This oxidant was shown to be a potent inducer of tissue factor (TF) expression by human umbilical vein endothelial cells, with associated stimulation of the NF-κB transcription factor pathway. By increasing TF expression on endothelial cells adjacent to sites of eosinophilic infiltration and activation, HOSCN may enhance activation of the extrinsic coagulation cascade, resulting in local thrombus formation. Another pro-coagulant effect of cationic proteins consists in their ability to directly activate platelets. Indeed, incubation of isolated platelets with MBP and EPO (but not ECP or EDN) resulted in significant release of 5-hydroxytryptamine and degranulation [14], qualifying these proteins as strong platelet agonists.

Finally, coagulation may be affected by eosinophil-expressed molecules other than cationic proteins. In vitro-activated eosinophils and eosinophils isolated from a patient with hypereosinophilic syndrome were shown to express functional CD40L [15], which is thought to enhance pro-coagulant properties of the endothelial surface through interactions with endothelial cell-expressed CD40. More recently, eosinophils themselves were shown to contain significant amounts of TF within intracellular granules, which became membrane-expressed after in vitro activation using PAF and GM-CSF [16]. Membrane-expressed TF was shown to co-localize with CD125 (the IL-5 receptor α-chain, specifically expressed by eosinophils) in skin biopsies from patients with bullous pemphigoid [17], establishing the in vivo relevance of these findings. Besides its effects on coagulation, TF expression by eosinophils favors their trans-endothelial migration and hence local inflammation.

Cardiovascular complications of hypereosinophilia and associated clinical manifestations

The cytotoxic and pro-coagulant properties of eosinophils account for the development of cardiovascular complications in a significant proportion of hypereosinophilic patients. In vitro data indicate that eosinophils must be activated to induce these alterations. Conditions favoring eosinophil activation are more likely encountered in tissues, through interactions with various immune and resident cells. It is therefore conceivable that endothelial cell damage and thrombosis develop more readily at sites of eosinophilic inflammation. However, activated eosinophils release large amounts of cationic proteins that are detectable (sometimes at substantial levels) in serum from patients with hypereosinophilia. These proteins have the capacity to bind to endothelial surfaces, and may therefore also be responsible for more distant and/or systemic (micro)vascular damage or intravascular coagulation [18], unrelated to local eosinophil infiltrates.

Clinically, the presence of splinter hemorrhages is thought to reflect a pro-thrombotic state in patients with hypereosinophilia, and this sign should be taken very seriously. The eosinophil target-organ that has nurtured the most interest is the heart, with numerous published reports and studies on the subject. Cardiac involvement typically evolves in three stages, although patients often present with some degree of overlap [2]. First, eosinophils infiltrate the myocardium and cause necrosis, generally causing little or no symptoms. Patients occasionally experience acute dilated cardiomyopathy and heart failure at this stage. Secondly, eosinophils damage the endothelial lining, favoring generation of eosinophil-rich intracavitary thrombi. Their presence in the left chambers may be complicated by peripheral embolization with ischemia. Third, with prolonged eosinophilic infiltration, endomyocardial fibrosis develops, leading to restrictive cardiomyopathy often associated with impaired valvular functioning.

Cerebrovascular ischemic events are also a very preoccupying consequence of persistent hypereosinophilia. In many instances, they occur secondarily to embolization of cardiac mural thrombi, although multiple cerebral infarcts in watershed zones have also been reported in the apparent absence of intracardiac material [19]. One group described progressive extension of cerebral ischemic zones on serial imaging studies, and associated clinical worsening over time, in a patient whose brain biopsy showed cerebral infarction and presence of intravascular eosinophils [19]. The authors suggested that in some cases, the initial event may be acute cerebral ischemia due to eosinophil-rich micro-emboli originating from the heart, followed by local deposition of eosinophil granule proteins which further contribute to microvascular damage and coagulation in the microenvironment, thereby increasing the extent of vessel occlusion.

Besides those irrigating the brain, other large to medium-sized arteries may be occluded due to peripheral embolization (or extension) of intracardiac thrombi, including coronary arteries, aorta, mesenteric arteries, and arteries in the limbs [20–22]. Peripheral ischemia may also develop independently of overt heart disease, with evidence for direct eosinophil-mediated (micro)vascular obliteration. Biopsies of occluded vessels may show eosinophils infiltrating the vessel wall (i.e. “vasculitis”) [23] and/or within the associated intravascular thrombus [24]. Deposition of MBP was observed in glomeruli and afferent arterioles, in association with nearby eosinophilic infiltrates, in two patients with HES who developed acute renal failure and thrombotic microangiopathy [25]. Clinically, involvement of small-sized arteries and the microvasculature may also present as digital necrosis, cutaneous necrotizing vasculitis, retinal vessel occlusion, or livid-doid discoloration of the skin [23,26,27], and it is likely that numerous small infarcts may occur in any organ, resulting in functional impairment.

Patients with persistent hypereosinophilia are also prone to develop venous thrombosis, with numerous reports of deep vein thrombosis and/or pulmonary embolism, Budd-Chiari syndrome, thrombosis of intracranial sinuses, mesenteric and portal veins, and superficial venous thrombophlebitis [28–32]. A single
case of hepatic veno-occlusive disease developing in a patient with hypereosinophilia has been reported [33], with reversal of associated clinical and biological perturbations in response to corticosteroid therapy.

The diagnostic and therapeutic management of cardiovascular complications of hypereosinophilia have recently been extensively reviewed [34], and will not be developed herein.

**Concluding remarks**

Eosinophil-mediated cardiovascular complications are a major determinant of morbidity and mortality associated with marked hypereosinophilia. Early therapeutic control of eosinophil counts is essential for prevention of these potentially devastating incidents. Unfortunately, thrombosis may occur relatively early in the course of hypereosinophilia, and a recent study has shown that in more than half of reported patients with HES and Churg-Strauss syndrome complicated by a thrombotic event, diagnosis of the hypereosinophilic condition was made after it occurred [35]. Rapid and effective therapeutic control of eosinophil counts, together with tightly controlled anticoagulant and antiplatelet treatment, are critical to limit the burden of cardiovascular complications of hypereosinophilia.

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**References**


Churg-Strauss syndrome (CSS) is the least common of the antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides, which also include microscopic polyangiitis and granulomatosis with polyangiitis (Wegener’s). CSS has been recently renamed as eosinophilic granulomatosis with polyangiitis (EGPA) (Churg-Strauss) [1].

Everlasting messages from history

The first well-described vasculitis in medical history was periarteritis nodosa in 1866, by Kussmaul and Maier, who reported “a hitherto undescribed peculiar disease of the arteries which is accompanied by Bright’s disease and a rapid progressive paralysis of the muscles” [2]. Thirteen years later, Ehrlich discovered the eosinophil leukocyte [3]. The first published case of EGPA was that of Albert Lamb in 1914 [4] under the heading of polyarteritis nodosa. The 26-year-old patient he reported had asthma, eosinophils up to 7.7 G/L, and he died 1 month after admission to the hospital. Autopsy disclosed especially myocarditis with periarterial eosinophilia and glomerulonephritis with epithelial crescents. Several reports and short series were further published in the following years, until Churg and Strauss [5] eventually reported a well-studied series of 13 cases with extended autopsy in nine. They emphasized the severity of asthma, and the manifestations of cardiac failure, renal damage, and nervous involvement. The characteristic histopathologic features that they described mainly consisted of necrotizing arteritis, eosinophil infiltration, and granulomatosis with giant cells. Up to the 1950s almost all patients with EGPA died and autopsy studies could thus describe the entity in detail.

After corticosteroids had become available, improvement in EGPA was obtained; however it was only in the 1980s that cytotoxic drugs (especially cyclophosphamide and azathioprine) were combined with corticosteroids, and that the prognosis of EGPA eventually improved with long-lasting remission and eventual healing.

Diagnostic criteria: a protracted challenge

Surprisingly, up to now no consensual diagnostic criteria have been established for EGPA. Lanham et al. [6] reported 16 cases and reviewed the literature based on diagnostic criteria including asthma, eosinophils greater than 1.5 G/L, and systemic vasculitis involving two or more extrapulmonary organs. Masi et al. [7] reported the American College of Rheumatology criteria for the classification of CSS which have often inappropriately been used as diagnostic criteria. This was quite inadequate, as for example a patient with idiopathic chronic eosinophilic pneumonia with asthma and paranasal sinusitis could fit such criteria and therefore be called CSS. Furthermore, the Chapel Hill nomenclature was convened as a nomenclature and neither as a classification system nor as a diagnostic system [8].

From nomenclature to diagnostic criteria

The 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides [1] decided to rename CSS as EGPA (Churg-Strauss), a terminology that reminds the user that EGPA is indeed a vasculitis.

In recent years, distinct phenotypes have emerged from the largest series of CSS [9,10] with a vasculitic phenotype (with patients having more frequently ANCA, glomerulonephritis, alveolar hemorrhage, and/or biopsy-proven vasculitis) and a tissular phenotype (with patients having more frequently cardiomyopathy, but no ANCA).

Members of the European Respiratory Society CSS-Task Force (listed below as authors) prepared recommendations for the diagnosis of EGPA, including a definition of detailed criteria of vasculitis (or surrogates of vasculitis) in patients with asthma and blood eosinophils greater than 1.5 G/L (box 1). The CSS-Task Force further proposed that patients with asthma and blood eosinophils greater than 1.5 G/L without ANCA, vasculitis, or surrogates of vasculitis be called hypereosinophilic asthma with systemic manifestations (HASM), non-vasculitic (box 2).