Pediatr Nephrol (2007) 22:1083–1094
DOI 10.1007/s00467-007-0450-1

REVIEW

# What's new in the aetiopathogenesis of vasculitis?

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Received: 12 December 2006 / Revised: 5 January 2007 / Accepted: 8 January 2007 / Published online: 15 March 2007


**Abstract** The cause of the majority of childhood vasculitides is unknown although it is likely that a complex interaction between environmental factors and inherited host responses trigger the disease and determine the vasculitis phenotype. Epidemiological clues continue to implicate infectious triggers in Kawasaki syndrome (KS) and Henoch Schőnlein purpura (HSP). Several genetic polymorphisms have now been described in KS and HSP which predispose to disease or predict disease severity. Anti-neutrophil cytoplasmic antibodies (ANCA) are now known to be directly involved in the pathogenesis of vascular injury in ANCA-associated vasculitides, although why some individuals develop ANCA in the first instance is not yet understood. Endothelial injury and repair are active areas of research in vasculitis. It is now possible to track endothelial injury non-invasively in children with vasculitis using surrogate markers of endothelial injury. The vasculogenic pathways involved in vascular repair following vasculitis, including endothelial progenitor cells, are beginning to be studied. It is anticipated that an improved understanding of the aetiopathogenesis of vasculitis in the young will ultimately shape future novel diagnostic and therapeutic approaches and will help us predict which children may develop premature arteriosclerosis in later life.

**Keywords** Aetiology · Child · Pathogenesis · Vasculitis

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## Introduction

Primary systemic vasculitis (PSV), while rare in children, is still associated with significant mortality and morbidity. Primary vasculitic syndromes include, amongst others, Henoch Schőnlein purpura (HSP), Kawasaki syndrome (KS), polyarteritis nodosa (PAN), Takayasu disease (TD) and the anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAV), comprising Wegener's Granulomatosis (WG), microscopic polyangiitis, renal limited vasculitis and Churg-Strauss syndrome [1]. PSV is characterised by the presence of inflammation in the walls of blood vessels, with resultant tissue ischaemia and necrosis [1]. The cause of the majority of childhood vasculitides is unknown, although it is likely that a complex interaction between environmental factors, such as infections and inherited host responses, trigger the disease and determine the vasculitis phenotype [2, 3] This review summarises the findings of recent studies relating to the aetiopathogenesis of PSV and will be considered under the two broad headings of environmental triggers and host responses. This article will not focus on the therapy of PSV, nor will the article describe comprehensively all of the possible infective organisms now known to be associated with secondary infective vasculitis (very recently reviewed by Pagnoux et al. [4] and Naides [5]).

## Environmental triggers

### Epidemiological clues

A number of important epidemiological studies have recently been published describing the demographics of

PSV in the young [6–8], but as yet these have failed to definitively cast light on the aetiology of PSV in children. Epidemiological data are most readily available for the two commonest forms of PSV in children, HSP and KS, with relatively few data relating to the epidemiology of PAN or AAV in children. Common themes emerging relate to seasonality and ethnic predisposition, in particular in relation to KS, hinting at infectious precipitants and/or genetically determined host-responses. KS is commonest in Japan and in Japanese and other Oriental children living abroad [9]. Children aged 6 months to 5 years are the most susceptible, with peak incidence in children aged 9–11 months [10, 11]. Seasonal variation in KS incidence has been reported, with peak occurrence in the winter and spring months [10, 11]. Outbreaks of KS have been linked to weather patterns, with clusters of KS cases occurring in association with precipitation [12]. Direct person-to-person spread has not been observed, although in Japan the disease occurs more commonly in siblings of index cases, with an estimated peak incidence of 8–9% in siblings under the age of 2 years [13]. Currently, the estimated incidence of KS in the UK is 8.1 per 100,000 children under the age of 5 years – an incidence which has doubled since 1991 [14]. While this increase may truly indicate an increasing incidence, better case recognition may partly account for this rising trend. Whilst these observations suggest an infectious aetiology, the hunt for any single infectious agent has so far not proved fruitful, a fact most recently highlighted by the negative results that emerged from studies examining the potential link between coronavirus infection and KS in Taiwan [15]. It is likely that KS represents an extreme endpoint of any number of potential infectious agents in a genetically predisposed individual. This has prompted the recent change of nomenclature from Kawasaki disease to Kawasaki syndrome, which serves to focus attention on host responses rather than specific infectious agents.

HSP, similar to KS, also appears to have a seasonal variation, occurring more commonly in the autumn and winter [7, 16]. Yang et al. commented that the clustering of HSP in the autumn and winter, often preceded by upper respiratory infections, points to the possibility that HSP is infection-related [7], possibly to inciting agents such as streptococci [17], *Bartonella henselae* [18], parvovirus [19], HIV [20] and varicella [21], amongst others. As in KS, however, no single infectious agent has been identified, and it is likely that genetically controlled host responses determine whether or not an individual develops HSP in response to infectious triggers.

Epidemiological studies of AAV in adults have provided important clues relating to other environmental and genetic factors (see below) which may be involved in the aetiopathogenesis of these syndromes. The geographic distribution of AAV disease occurrence in adults suggests that WG occurs more commonly in northern European countries than in southern European countries, with a twofold higher annual incidence being observed in Norway and England than in Lugo, Spain [22, 23]. The human leukocyte antigen (HLA) make-up of the Lugo population differed from that found in other Mediterranean areas and had a similar proportion of HLA-DRB1 alleles to that found in the UK population. Since no clear HLA association has been shown with any type of PSV other than Behcet's disease, Watts et al. suggest that environmental factors may be more important than genetic factors in WG [22, 23].

In this context there is a picture emerging of a potential relationship between occupational crystalline silica exposure and AAV that has been observed in a number of studies [24–28]. Overall exposure to silicon-containing compounds was found to be related to AAV, particularly WG, with an odds ratio of 2.5–5 [25–28]. The mechanisms by which silica may induce AAV are not well known. Silicon-containing compounds may be immune adjuvants, since silica particles are potent stimulators of lymphocytes, monocytes and macrophages. Silica particles may also stimulate neutrophil-reactive oxygen radical release and inflammatory cytokines, triggering exocytosis of the proteinase 3 and myeloperoxidase antigens on the surface of the polynuclear neutrophils, thus priming neutrophils for ANCA activation (see below). Whether this risk factor is important in WG in children or young adults is, however, questionable.

Other miscellaneous environmental risk factors for AAV in adults have been reported, such as smoking, which has been found by some researchers to have either a protective effect [29] or no effect at all [30, 31], putative triggering of WG by drug exposure, notably propylthiouracil or hydralazine [32], and links between malignancy and onset of WG [33]. These factors are, however, likely to be of less relevance as risk factors for paediatric AAV.

## Infections and PSV

Many pathogens are known to either directly cause vasculitis or to act as potential triggers for PSV [4, 5]. Many of the viruses, bacteria and fungi that directly cause vasculitis demonstrate tissue tropism that includes the endothelium; other agents may bind to the vessel wall because the vascular endothelium expresses specific receptors for the pathogen. Even when the agent does not enter the endothelial cell, the immune response to the agent may still be focused at the endothelium because the pathogen is adherent to the endothelial cell surface, resulting in bystander injury to the endothelium and vessel.

## Superantigens and PSV

Superantigens (SAgs) are one of the environmental factors that have been proposed to modulate a number of autoimmune diseases, including vasculitis. The most compelling evidence for the involvement of SAg in the pathogenesis of vasculitis relates to KS, although this hypothesis has proved to be controversial [34]. SAgs are a class of immuno-stimulatory proteins of bacterial or viral origin with the ability to activate large fractions (5–30%) of the T cell population, and they are responsible for human toxic shock syndrome and some forms of gastroenteritis [3, 35]. Class II major histocompatibility complex (MHC)-positive endothelial cells operate as competent SAg-presenting cells for CD4 and CD8 lymphocytes in vitro. Dual signalling between endothelial cells and T cells results in Vβ-restricted activation and adherence to endothelium, resulting in endothelial cell activation and injury [3]. Thus, SAgs could mechanistically result in severe endothelial and vascular injury and could account, in part, for the vascular injury associated with SAg-mediated diseases such as toxic shock syndrome.

One important controversial issue is the ongoing debate regarding SAgs versus conventional antigens in the aetiopathogenesis of KS. There are similarities between the clinical and immunological features of KS and SAg toxin-mediated staphylococcal and streptococcal toxic shock syndromes, and scarlet fever [3].

The study by Abe et al. in 1992 was the first to describe the selective expansion of Vβ2 and Vβ8.1 T cells in KS [36], indicating T cell Vβ skewing – the hallmark of a SAg-mediated process. Since then, many similar studies have examined T cell Vβ repertoires in KS, or examined the prevalence of serological conversion or colonisation with SAg–producing organisms [37, 38]. Another important observation is that T cells infiltrating the walls of coronary arterial aneurysms and the intestinal mucosa of patients with KS show a skewed T cell Vβ repertoire, with increased numbers of cells expressing Vβ2 [39, 40], again possibly indicative of a SAg-driven process.

However, Rowley et al. recently reported three fatal cases of KD and observed IgA plasma cell infiltration into the vascular wall during the acute phase of the illness. By examining the clonality of this IgA response using reverse transcriptase (RT)-PCR in lesional vascular tissue, these researchers observed that the IgA response was oligoclonal, suggesting a conventional Ag process rather than a SAg-driven one [41]. Synthetic versions of the prevalent IgA antibodies were then used in immunohistochemical experiments on acute KS and control tissues; several of the most prevalent IgA antibodies detected antigen in lesional KS tissue, but not in control tissue. Further studies indicated that the antigen resides in cytoplasmic inclusion bodies [42], which is possibly consistent with aggregates of viral protein and nucleic acid. These important observations may suggest that there is a common infectious aetiology for KS, perhaps viral, and may cast doubt on the SAg hypothesis in KS.

We recently described the T cell Vβ repertoires in a number of paediatric vasculitic syndromes [2]. Like others, we found evidence of Vβ2 expansions in peripheral blood of KS patients and skewing of the peripheral blood T cell Vβ repertoire in 25 children with PSV, predominantly PAN. No such skewing of the Vβ repertoire was observed in HSP patients. While these data provide impetus for further research into this contentious field, they do not resolve unequivocally the question of the role of SAgs in childhood vasculitic syndromes. That said, a recent multi-center review of Turkish children with PAN again has implicated streptococcal infection in the aetiopathogenesis of this vasculitic syndrome. Whether the mechanism is that of molecular mimicry of a conventional antigen or superantigenic-mediated vascular injury is unknown [43]. Thus, the jury remains out on the SAg hypothesis in PSV of the young.

## *Staphylococcus aureus* and WG

Several groups have been actively researching the relationship between *Staphylococcus aureus* and WG [44]. There is an increased incidence of chronic nasal carriage of *S. aureus* in patients with WG, and this is associated with an increased risk of disease relapse [45]. Moreover relapses of WG can be reduced by the use of long-term trimethoprim-sulphamethoxazole [46], although it is currently unclear whether this efficacy results from eradication of the nasal carriage of *S. aureus* or by virtue of a potential anti-inflammatory effect of trimethoprim-sulphamethoxazole, which exerts its antibiotic effect by antagonising folic acid metabolism, a mechanism which could affect leucocytes. Possible mechanisms whereby *S. aureus* could result in flares of WG include SAg production and T and B cell activation, direct tropism of *S. aureus* for endothelial cells, with binding and internalisation of the organism by endothelial cells, or by priming of neutrophils (see below) [44]. Of note, however, is the observation that the carriage of staphylococcal strains that are positive for SAg genes in WG patients is not associated with the expected pattern of SAg-reactive T cells [47]. Of all these proposed mechanisms, the data currently suggest that the effects of *S. aureus* on endothelial cells and/or phagocytic cells may be the most plausible, with relatively scant evidence of a SAg effect based on studies of patterns of T cell Vβ skewing.

## Host responses

### Genetic predisposition to vasculitides

Several investigators have examined potential genetic factors predisposing to vasculitis. Familial cases of vasculitis including PAN [48], KS [49], AAV [50] and TD [51] have been described. It is clear from these studies, however, that the predisposing genetic factors of PSV are complex and multiple and that they vary between different vasculitic syndromes. Previously genetic studies had focused on AAV, although a number of publications have recently appeared that report on important mutations and/or polymorphisms in KS and HSP.

### KS and novel genetic associations

Biezeveld et al. recently reported on the association of a mannose-binding lectin (MBL) genotype with cardiovascular abnormalities in KS [52, 53]. MBL is a macromolecule with collagenous and sugar-binding domains and is able to activate the complement pathway directly (i.e. independent of antibodies). MBL is thus the "third" limb of the complement cascade and is termed the MBL pathway. In particular, MBL binds to repeats of N-acetyl-glucosamine and mannose residues, expressed on the surface of many microbial antigens. A deficiency of MBL (found in up to a third of the healthy population) is associated with an increased risk of bacterial and viral infection in children [54]. Thus, MBL is an important component of the innate immune system, particularly in the acute phase of infections. Infants in the period between decreasing levels of maternal antibodies and the development of adaptive immunity are especially dependent on this molecule. In two studies of 90 Caucasian children with KS in The Netherlands, Biezeveld et al. observed what appears to be an ambiguous role for MBL in this context [52]. Infants with KS under 1 year of age were at higher risk of developing coronary artery aneurysms (CAA) if polymorphisms in the *MBL2* gene (correlating with low plasma MBL levels) were present (odds ratio: 15.7; 95% confidence interval: 1.4–176.5). Over the age of 1 year the reverse was true: the wild-type MBL genotype and normal plasma MBL levels were associated with a greater risk of CAA [53]. Thus, it appears that MBL modulates host responses and cardiovascular injury differently depending on age. In infants under the age of 1 year, normal MBL levels may protect from any putative infective organism triggering vascular injury in KS; whereas in older children who are not critically dependent on MBL for innate immunity, MBL, itself an acute phase protein, could contribute to endothelial injury by uncontrolled complement activation [53].

To date, there are only limited data examining the role of the MBL genotype in other vasculitides. Endo et al. detected the glomerular deposition of MBL and MBL-associated serine protease (MASP-1) as well as C3b/C3c, C5b-9, and C4-binding protein (C4-bp) in eight of ten patients with HSP nephritis, suggesting a role for MBL in glomerular injury associated with HSP [55].

Mutations and polymorphisms in several other miscellaneous genes have recently been described in KS, either as disease-susceptibility genes influencing the risk for CAA development or as a response to treatment. These are summarised in Table 1.

### Genetic polymorphisms and HSP

Several polymorphisms relating to disease susceptibility, severity and/or risk of renal involvement have recently been described. Many of these polymorphisms relate to cytokines or cell adhesion molecules involved in the modulation of inflammatory responses and endothelial cell activation, and may therefore have relevance in other autoimmune diseases as well as HSP. On the whole, studies of this nature have been hampered by relatively small patient numbers and thus lack the power to be definitive or necessarily applicable to all racial groups. These studies are summarised in Table 1.

### Genetic polymorphisms and ANCA associated vasculitides

A plethora of studies have examined multiple putative genes that may be involved in the aetiopathogenesis of AAV. These are summarised in Table 1.

### Autoantibodies and vascular injury: ANCA

Only recently has the evidence that ANCA are directly involved in the pathogenesis of vascular injury been documented. The most compelling evidence are reports of two neonates who developed pulmonary renal syndrome soon after birth from the trans-placental transfer of IgG MPO-ANCA from mothers with active microscopic polyangiitis (MPA) [56, 57]. Moreover, a mouse model recently described by Xiao et al. showed that the passive transfer of MPO-ANCA or anti-MPO lymphocytes (that make MPO-ANCA) into mice that lack functioning B or T cells (RAG −/−) resulted in pauci-immune crescentic nephritis and small vessel vasculitis similar to that observed in humans with AAV [58]. Thus, the evidence that MPO-ANCA are directly involved in the pathogenesis of vascular injury is growing, but may still be somewhat debated for PR3-ANCA.

A plethora of publications describe in detail the mechanism of vascular injury mediated by ANCA, and

**Table 1** Genetic polymorphisms studied in KS, HSP, and AAV[a]

| Molecule/genetic polymorphism | Vasculitis type | | |
|---|---|---|---|
| | KS | HSP | AAV |
| Mannose binding lectin (MBL) | Ambiguous role for MBL influencing risk of coronary artery aneurysms (CAA); see main text | MBL and MBL-associated serine protease (MASP-1) detected in glomerular lesions of HSP- see main text | Not studied |
| Angiotensin converting enzyme (ACE) | ACE I/D polymorphism increases disease susceptibility [106] | No association of HSP nephritis with polymorphisms in ACE, albeit in studies involving small numbers of children [107–109] | Not studied |
| Matrix metalloproteinases (MMP) | MMP-3 6A/6A polymorphism results in higher frequency of CAA [110] | Genetics not studied; MMP-9 may be elevated in HSP [111] | Genetics not studied; MMPs expressed in lesional glomerular tissue [112] |
| Vascular endothelial growth factor (VEGF) and its receptor (KDR) | Polymorphisms of both contribute to increased CAA risk [113] | VEGF polymorphisms predispose to renal involvement [114] | Genetics not studied; VEGF elevated in active WG [115] |
| Interleukin 1 receptor antagonist (IL-1Ra) | Polymorphism associated with increased disease susceptibility [116] | Polymorphism predisposes to renal involvement [117] | Not studied |
| Interleukin 1β (IL-1β) [118] | No association found [116] | Polymorphism associated with renal involvement [118] | One study failed to identify associations between IL-1β polymorphisms and WG [119] |
| Tumour necrosis factor-alpha (TNF-α) | TNF-α-308A associated with increased intravenous immune globulin (IVIG) resistance [120] | TNF-alpha G-308A polymorphism not associated with HSP in Chinese patients [121] | One study failed to identify associations between TNFα polymorphisms and WG [119] |
| Interleukin-8 (IL-8) | Genetics not studied | Polymorphism associated with renal involvement [122] | Genetics not studied |
| Interleukin-10 (IL-10) | IL-10 gene promoter polymorphisms influence risk of CAA [120] | Genetics not studied | IL-10 (−1082) polymorphism associated with WG and MPA [123] |
| Chemokines | Chemokine receptor CCR5 and its ligand CCL3L1 influence disease susceptibility [124] | Genetics not studied | Genetics not studied |
| Familial Mediterranean Fever genotypes (MEFV gene mutation) | Genetics not studied | Mutations in MEFV found more commonly in Israeli and Turkish children with HSP [125, 126] | Genetics not studied |
| Human Leucocyte Antigens (HLA) | No consistent associations | Positivity for HLA-B35 predisposes to renal involvement in HSP [127] | No consistent associations |
| PAX2 (Paired box gene 2) | Genetics not studied | Polymorphisms in PAX2 predispose to renal involvement in HSP [128] | Genetics not studied |
| Nitric oxide and associated molecules | No association of ecNOS and iNOS gene polymorphisms to the development of CAL in Japanese KS patients [129] | Inducible nitric oxide synthase 2A promoter polymorphism predisposes to renal involvement [130] | Genetics not studied |
| Cell adhesion molecules | Genetics not yet studied | Patients not carrying the codon ICAM-1 469 K/E genotype are at decreased risk of developing severe gastrointestinal complications [131] | Polymorphism in exon 11 of the CD18 gene associated with MPO-ANCA vasculitis; no relevant polymorphisms were identified for ICAM-1, E-selectin, CD11b, or human urokinase plasminogen activator receptor gene [132] |

**Table 1** (continued)

| Molecule/genetic polymorphism | Vasculitis type | | |
|---|---|---|---|
| | KS | HSP | AAV |
| α-1-Antitrypsin | Genetics not studied | Isolated case reports of severe multi-systemic HSP and α-1-antitrypsin deficiency [133] | An association between PR3-ANCA and the deficient PiZZ phenotype has been described [134, 135]. Alpha-1-antitrypsin deficiency is not sufficient in itself to cause ANCA-positive vasculitides, but may act as an amplifying factor [136, 137] |
| Proteinase 3 (PR3) | Genetics not studied | Genetics not studied | Association with the A-564G polymorphism in the proteinase-3 promoter and WG [138] |
| Fcγ receptors | No association for FcgammaRIIa-131H/R, FcgammaRIIb-232I/T, FcgammaRIIIa-158 V/F and FcgammaRIIIb-NA1/NA2 [139] | Genetics not studied | Possible association between NA1 allele of FcγRIIIb in patients with WG and renal involvement[140]. FcγRII-R131 and FcγRIIIa-F158 may predispose to disease relapse [141]. |
| CTLA-4 (cytotoxic T lymphocyte-associated antigen-4) | Genetics not studied | Genetics not studied | Polymorphism associated with WG [119] |

[a] KS, Kawasaki Syndrome; HSP, Henoch Schőnlein purpura; AAV, anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitides

these have been eloquently summarised in recent reviews [59, 60]. The most accepted model of pathogenesis proposes that ANCA activate cytokine-primed neutrophils, leading to bystander damage of endothelial cells and an escalation of inflammation with recruitment of mononuclear cells [61]. Neutrophil priming involves stimulation with an agent, such as lipopolysaccharide (LPS) or the cytokine tumour necrosis factor alpha (TNF-α), at a concentration that does not of itself cause full functional activation but rather results in the translocation of ANCA-antigen (MPO and/or PR3) from intracellular granules to the cell surface. TNF-α priming and subsequent ANCA activation of neutrophils lead to a respiratory burst and degranulation, thereby generating the potential for endothelial injury. The binding of ANCA to antigens expressed on the surface of primed neutrophils is not enough in itself to cause full neutrophil activation; the binding of Fc receptors is also necessary [62]. Following neutrophil stimulation by ANCA, numerous cytotoxic mediators are released, including reactive oxygen species, chemokines, cytokines, proteolytic enzymes and nitric oxide (NO) [63]. The firm adhesion of activated neutrophils to endothelial cells results in endothelial cell damage and perhaps recruitment of other inflammatory cells including monocytes and T cells [64]. Following neutrophil activation by ANCA, neutrophils are driven down an accelerated but aberrant apoptotic pathway [65]. Thus, the neutrophils will develop the morphologic features of apoptosis, but there is a failure in cell surface changes, such as bi-lipid cellular membrane phosphatidylserine (PS) externalisation [66, 67] , which is normally an early feature of all apoptotic cells. This lack of PS externalisation means that phagocytes are less able to process the apoptotic neutrophils in a non-phlogistic fashion, explaining the well-characterised finding of leucocytoclasis that is often seen in the vasculitic lesions of certain vasculitic syndromes.

## Autoantigen complementarity and AAV

The aforementioned clinical and experimental evidence support a role for ANCA in the pathogenesis of AAV, but it does not explain why ANCA develop in certain individuals. One emerging hypothesis which could cast light on this area is termed the complementarity theory [68]. The central premise for this theory is that anti-PR3 autoantibodies (PR3-ANCA) are generated by an immune response that initially is mounted against a peptide that is antisense or "complementary" to the autoantigen [68]. The implication is that infectious agents could trigger ANCA generation via microbial peptides which act as molecular mimics for complementary PR3. Indeed *S. aureus* contains a peptide sequence that mimics antisense PR3, as do the Ross River virus and *Entamoeba histolytica*, which are all infections previously associated with ANCA generation in

humans [69]. The theory of autoantigen complementarity could thus have important implications for the pathogenesis of other autoimmune diseases, but this needs further validation.

## Downstream mediators of vascular injury

A number of studies have looked at other biomarkers predictive of CAA in KS. Two biomarkers deserve particular mention in this context: the matrix metalloproteinases (MMPs) and the S-100 proteins.

An imbalance between the MMPs and the tissue inhibitor of MMP (TIMP) has been implicated in the development of CAA in KS [70]. The extracellular matrix is maintained by a rigorously controlled balance between the synthesis and breakdown of its component proteins. MMPs and TIMPs play central roles in this process. Children with KS and high levels of MMP and/or a high MMP/TIMP ratio are more susceptible to coronary arterial lesions [71].

Calcium-binding proteins in the S-100 family, MRP-8 and MRP-14, are potential biomarkers for KS and CAA. These proteins form heterodimers and are secreted by neutrophils and monocytes in response to inflammatory signalling cascades. The MRP8/MRP14 heterodimer binds to microvascular endothelial cells and may participate in the genesis of a proinflammatory and prothrombotic state during systemic vasculitis [72]. Specifically, these proteins regulate the adhesion of neutrophils and monocytes to endothelial cells and are implicated in their transmigration into the vessel wall. Serum MRP-8/MRP-14 levels as well as mRNA expressions of MRP-8 and -14 in granulocytes were upregulated in acute KS and decreased dramatically within 24 h of intravenous immune globulin (IVIG) therapy [72]. The number of MRP-8/MRP-14-positive circulating endothelial cells (CECs, see below) was higher in patients with acute KD than in control patients, and the numbers increased significantly by 2 weeks after the onset of KD, especially in patients in whom coronary artery lesions developed [72].

One important implication of these observations is that these pathways could provide new therapeutic targets for IVIG non-responders or those with severe CAA and partial IVIG response.

## Tumour necrosis factor alpha and vasculitis

There is much interest in the role of TNF-α as a downstream mediator of endothelial and vascular injury and the potential for a therapeutic blockade of this cytokine in a number of paediatric and adult vasculitic syndromes [73, 74]. The evidence for efficacy of TNF-α blockade in paediatric vasculitis currently remains anecdotal – albeit promising – and an important addition to the armamentarium for diseases recalcitrant to conventional therapy [75, 76].

## Tracking endothelial injury in vasculitis

A number of groups have recently described methods for detecting endothelial cell components in blood which may allow the assessment of the molecular events associated with vascular injury. Two potentially informative methods have been reported: endothelial microparticles (EMPs) and circulating detached mature endothelial cells (CECs).

EMPs are released from the cell surface in response to a variety of stimuli associated with endothelial injury, including vasculitis [77], atherosclerosis and acute coronary events [78] and thrombotic thrombocytopenic purpura [79]. We have previously demonstrated that it is possible to monitor endothelial injury in vasculitis in children through the detection of circulating EMPs expressing E-selectin, or CD105, in effect providing a window to the activated endothelium [80].

CECs are necrotic or highly activated endothelial cells which detach from the vessel wall [81]. They have recently been shown to be a useful clinical marker of endothelial injury in vasculitis in adults [82–84], stroke [85] and pulmonary hypertension [86]. Woywodt et al. suggest that CECs correlate with disease activity in adults with ANCA vasculitis and are thus useful both diagnostically and also for monitoring disease activity in response to therapy [84]. Our preliminary data (PB et al., unpublished) suggest that CECs are also elevated in children with active primary systemic vasculitis and that they may also be used as a novel clinical tool to monitor disease activity in response to treatment.

## Vascular repair mechanisms

A recently emerging concept now supported by several animal and human studies is that bone marrow-derived endothelial progenitor cells (EPCs) are involved in both normal physiological and pathophysiological repair mechanisms, for example during myocardial vascular regeneration following myocardial infarction and in post-transplant arteriosclerosis [85–87]. The discovery of EPCs by Asahara et al. represented a major advance which has altered our understanding of endothelial repair and the process of neovascularisation and focused considerable research interest in these areas [88]. These researchers showed that CD34 + peripheral blood mononuclear cells from healthy adults acquire an endothelial cell-like phenotype in vitro and are incorporated into regenerating capillaries in a murine hindlimb ischaemia model, findings subsequently confirmed by several groups.

The number and the function of circulating EPCs influence cardiovascular risk and endothelial responses to insults associated with endothelial injury in humans. Disease and pre-disease states studied to date include: conventional atherosclerotic risk factors [87, 89]; connective tissue/inflammatory diseases, including rheumatoid arthritis [90]; systemic sclerosis [91]; Wegener's granulomatosis [92]; Kawasaki disease [93] (although data relating to children are limited to this latter small study). EPCs are known to originate in the bone marrow, and they function normally via homing and adherence to regions of damage [94, 95]. EPC responses to the treatment of vasculitis in children have yet to be studied, but it is an area of ongoing active research.

### Does vasculitis in the young predispose to premature atherosclerosis?

Several key aspects of the long-term outcome of vasculitis in the young remain of ongoing concern and are currently being best studied in KS. Most importantly, controversy continues as to whether KS constitutes a risk factor for premature atherosclerosis. Histological findings seen in KS arteries at sites of previous aneurismal lesions long after disease resolution appear to be indistinguishable from atherosclerosis [96]. Dhillon et al. studied vascular responses to reactive hyperaemia in the brachial artery using high-resolution ultrasound [97]. Flow-mediated dilation (an endothelial-dependent response) was reduced in KS patients compared with control subjects many years after the illness, even in patients without detectable early coronary artery involvement. In a long-term case control study, Albisetti et al. showed that KS patients were more likely to have impairment of the fibrinolytic system, another marker of endothelial dysfunction, and this again was unrelated to the degree of coronary artery involvement [98]. Pilla et al. demonstrated reduced arterial distensibility (an independent risk factor for cardiovascular morbidity and mortality in adults), as assessed using ultrasound pulse wave velocity in the brachio-radial arterial segments of 43 children who had KD a median of 3 years previously [99]; this finding has been recently confirmed by Cheung et al. [100] and also documented in children with PAN [101].

In contrast, another recent cross-sectional study by McCrindle failed to demonstrate differences in brachial artery reactivity or carotid intima-media thickness following KS, although there was some degree of impaired blood pressure control documented on 24-h ambulatory blood pressure monitoring in KD cases [102].

Perhaps more important than these studies of novel surrogate markers for vascular injury are the long-term epidemiological data from Japan. In the fifth look at long-term outcomes of a cohort of 6576 patients with KD enrolled between 1982 and 1992, the mortality rate for patients without cardiac sequelae in the acute phase of the disease and female patients with sequelae did not differ from that of the normal population [103]. The mortality rate of males with cardiac sequelae was, however, 2.4-fold higher than that of the normal population. Thus, the long-term outlook for patients with coronary involvement due to KS, particularly males, must remain guarded at the present time.

## Conclusion and future directions

This is an exciting time for vasculitis research. Our understanding of the pathogenesis is advancing, as is our approach to novel therapeutic approaches, including the increasing use of biologics, although formal clinical trials involving children are still lacking in this area. Significant other challenges are looming. These include establishing international research networks and databases to obtain adequate patient numbers, thereby enabling powering of studies; validation of classification criteria suitable for the paediatric vasculitides [104, 105]; the development of tools to allow reliable non-invasive monitoring of disease activity; and the development of robust core outcome variables that can be used to assess outcomes in clinical trials. Lastly, there remains the question regarding longer term cardiovascular morbidity in children who survive vasculitis. Ultimately it must be anticipated that advances in our understanding of the environmental triggers and host responses will shape future novel therapeutic approaches to PSV in the young.

**Acknowledgments** I would like to thank Professor Michael Dillon for helpful comments on this manuscript.

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