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HSP60 and Anti-HSP60 Antibodies in Vasculitis: They are Two of a Kind

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Abstract Clinical and pathological manifestations present as heterogeneous in vasculitides. Thus, inflammation can affect arteries, arterioles, capillaries, venules, and veins toward major body regions. One common feature of vascular diseases appears to be the presence of anti-HSP60 autoantibodies arising either consecutively to infection and molecular mimicry reaction with bacterial HSP60, or following recognition of endogenous HSP60 translocated or bound onto the surface of stressed endothelial cells. Because their levels are very low in some diseases but strikingly upregulated in others, and because their frequencies vary from one vasculitis to another, anti-HSP60 autoantibodies might play a role in the pathological mechanisms that likely differ among the vascular diseases. Identification of the variety of HSP60 epitope specificities along with each vasculitis would help to understand such discrepancies.

Keywords Antibodies · Vasculitis · Endothelial cells · HSP60

Vasculitis

Definition

In essence, vasculitis reflects inflammation of the blood vessel walls. The implication is that endothelial cells (ECs) lining all vessels may stand as foremost inflammatory cells by triggering the process caused by primary perturbations, or behaving as targets because of secondary damages. Depending on the site and the type of blood vessels affected, clinical and pathological manifestations vary considerably. Such awareness has justified several nomenclatures, among which is the Chapel Hill nomenclature [1]. These entities may be autonomous and referred to as primary vasculitides, or be set against a background of autoimmune diseases and designated secondary vasculitides [2] The primary forms of the disease result from vasculitis, which is the triggering abnormality. They may affect large vessels in giant cell arteritis, Behcet's disease (BD) and Takayasu's arteritis (TA), medium vessels in polyarteritis nodosa (PAN) and Kawasaki disease (KD), or small vessels. Some of the latter conditions such as Wegener's granulomatosis (WG), microscopic polyangiitis and Churg–Strauss syndrome, are associated with specific antibodies (Abs) collectively coined antineutrophil cytoplasmic Abs (ANCAs), whereas others, such as Henoch–Schönlein purpura, mixed cryoglobulinemia and Goodpasture's disease, are not. With regard to secondary vasculitides, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren's syndrome (SS), or polymyositis (PM) may be at the forefront.

Anti-Endothelial Cell Antibodies

In light of the above findings, it is not surprising that vascular inflammation is frequently associated with anti-endothelial cell antibodies (AECAs). These may be the consequence of inflammatory process, and/or the cause of its expansion [3]. They have been detected in the sera of patients developing vasculitides and systemic autoimmune diseases (AIDs) at frequencies that vary from patient to

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patient in a given disease, and from disease to disease in a given patient (Table 1, and the article by Mouthon et al. in this issue).

By definition, AECAs react with ECs. Despite extensive studies, their antigen (Ag) specificity remains poorly identified. AutoAg can be structural molecules, *i.e.*, constitutively expressed on the cell membrane, or "planted Ags", *i.e.*, molecules bound to the EC membrane [4].

HSP60 as an Autoantigen

Function

A 60-kDa member of the heat-shock protein family called HSP60 has recently been claimed to be a target Ag for a subset of AECAs [5, 6]. This protein is involved in the folding, translocation, and assembly of proteins throughout their posttranslational maturation. In normal cells, endogenous expression of HSP60 is extremely weak at rest, but heavily induced following perception of danger signal like stress. Among numerous stresses are heat stress, oxidized low-density lipoproteins, biomechanical offense, infections, oxidants, and cytokines [7].

Table 1 Anti-endothelial cell and anti-HSP60 antibodies in vasculitis

Disease	AECA prevalence	Anti-HSP60 prevalence[a]
Primary systemic vasculitides		
Large vessels		
Giant cell arteritis	50% [48]	ND
Behçet's disease	75%	22–44%
Takayasu's arteritis	95% [49]	86%
Medium vessels		
Polyarteritis nodosa	53%	42–79%
Kawasaki disease	65% [50]	ND
Small vessels with ANCA		
Wegener's granulomatosis	80%	44–56%
Microscopic polyangiitis	50%	25–62%
Churg–Strauss disease	62%	0–20%
Small vessels without ANCA		
Herroch–Schönlein purpura	45% [51]	ND
Mixed cryoglobulinemia	41%	ND
Goodpasture's disease	ND	ND
Secondary systemic vasculitides		
Systemic lupus erythematosus	74%	39–76%
Sjögren's syndrome	24%	10–20%
Rheumatoid Arthritis	80%	10–20%
Polymyositis	38%	30%

[a] Depending on the technique as detection.
ND: Not determined

Cell Surface-expressed Molecule

In response to a stress, the target Ag HSP60 may be translocated to the outer leaflet of the surface of arterial [8] and venous [9] EC plasma membrane. The mechanism by which this exposition takes place remains uncharacterized.

Following a stress, HSP60 can be released by given ECs, and subsequently captured by the same or by other ECs [6]. That is, the soluble form of HSP60 could reflect its shedding from its intracellular compartments by damaged ECs. However, this process seems to be restricted to necrotic cells that gradually enhance their HSP60 expression [10], and absent from apoptotic cells [11], in spite of their release of HSP60 upon a danger signal [12]. Conceivably, depending on local environmental signals (danger, stress…), ECs can thus be induced to enhance the surface expression of HSP60 and release its soluble form. In this respect, the pathways must be unconventional [13] because HSP60 lacks the conventional signaling sequence for secretion.

HSP60 and Anti-HSP60 in Vasculitis

Primary Systemic Vasculitides

Large-sized Vasculitis

Behcet's disease (BD) is a systemic inflammatory disease of unknown aetiology. There have been repeated attempts to specify the role of HSP60 especially in BD. Serum levels of HSP60 in this disease are elevated, compared with the normal controls [14]. Although these levels do not correlate with disease activity, HSP60 appears to play an important role in the pathogenesis of BD, given the HSP60-induced activation of specific T cells. This may be caused by the contribution of Th1-dominant immune response [15, 16] against HSP60. These cells are aberrantly expressed in the peripheral blood lymphocytes and the intestinal lesions. To explain such findings, one may speculate that HSP60 may trigger, and/or be a target of the Th1 response. Because HSP60 shares common epitopes with bacterial HSP65, the inflammatory response may be ascribed to a molecular mimicry [17]. Interestingly, the p336–351 BD-specific peptide linked to recombinant cholera toxin B subunit protects five of eight patients from relapses when administered orally [18]. Clinical improvement was associated with a lack of peptide-specific CD4 T-cell proliferation, and a decrease in the number of Th1 cells. HSP60 is hence directly involved in the pathogenesis of the disease, and oral tolerization envisaged as an alternate therapy.

Furthermore, anti-HSP60 Abs have been reported in BD [19], as frequently as 22%, by enzyme-linked immunosorbent assay (ELISA), and 44% by Western (WB), compared

with healthy controls [6]. Reactivity towards *Streptococcus pyogenes* and bovine retinal HSP60s was also significantly increased [20], and ELISA identifies specificities shared by different HSP60s, and detects additional specificities in the bulk of anti-HSP60 Abs, which vary from one disease to another. These observations suggest that cross-reactivity between retinol and streptococcal HSP60s exists, so that anti-HSP60 autoAb response is suspicious in the development of the BD.

Takayasu's arteritis (TA) affects also the large vessels. Its aetiology remains unknown. Recently, it has been reported that CD4 T cells proliferate in the presence of HSP60, and the anti-HSP60 Ab test is positive in 84% of patients with TA. Significant correlations were found of HSP60-reactive T cells with mycobacterial HSP65-reactive T cells and of anti-HSP60 Ab with anti-HSP65 Ab [21]. Again, molecular mimicry could, therefore, be incriminated as a triggering agent of autoimmunity in TA. Furthermore, AECAs directed against aortic ECs have been shown to recognize HSP60 in 86% of patients with TA. These Abs were able to lift the surface expression of adhesion molecules, to increase the production of inflammatory cytokines, and to favor the apoptotic response of ECs [22]. They may thus also participate in the vascular dysfunction in this disease.

Medium-sized Vessel Vasculitis

One study shows higher amounts of HSP60 in lymphocytes of KD patients in acute phase, compared with those in convalescent phase. The plasma levels were, however, similar [23]. Although anti-HSP60 Abs have never been described in KD, the authors of this article suggest that under peculiar stimulation, HSP60 could be exposed on plasma membrane and generate these autoAbs. Consistent with this speculation are the finding of an upregulated expression of HSP60 on monocytes from patients in the acute phase of the disease compared with those in the subacute phase, and the detection of higher serum levels of HSP60 in the latter phase than in the former [24]. The inference in KD is that HSP60 could regulate the control of inflammation, instead of contributing to the inflammation. However, it has been reported that almost 30% of KD sera, in contrast to controls, recognize proteins of molecular mass corresponding to the cognate Ag of rabbit anti-HSP60 Ab [25]. Although, the nature of the epitopes and the consequences of Ab binding await determination, such reactivity could be increased with the susceptibility to the disease [26].

Small-sized Vessel Vasculitis

In contrast to the aforementioned studies, little data are available with respect to HSP60 and related autoAb in small-sized vessel vasculitides. In the Chapel Hill classification, a distinction is based on the presence or absence of ANCAs. Anti-HSP60 levels have recently been analyzed in patients with ANCA-associated vasculitis. These investigators failed to find significant differences between patient and control sera [27]. However, the anti-HSP60 level was increased in myeloperoxidase (MPO)-ANCA-positive patients compared with those positive for proteinase-3-ANCA, although neither correlation nor cross-reactivity could be observed between anti-HSP60 and MPO-ANCA. These data are inconsistent with the infection-derived trigger of an ANCA response against MPO through HSP60 cross-reactivity.

Vasculitis in Autoimmune Diseases (AIDs)

Homology between bacterial HSP65 and human HSP60 has shed some light on the molecular mimicry of the autoimmune traits to the infectious agents [28, 29]. This attractive hypothesis provides the impetus for numerous studies dedicated to the determination of the frequency of anti-HSP60 autoAbs in various autoimmune diseases (AIDs).

In SLE, the prototypic systemic autoimmune disease, HSP60 appears as the main Ag targeted [5]. However, depending on the Ag used in the assays and the method of detection, titer and frequency of anti-HSP60 autoAb vary from one study to another. Whereas serum concentration of anti-HSP60 did not differ from controls, in an ELISA based on the recognition of 0.1 μg recombinant HSP60 [30], the IgG titer was higher in SLE patient than in controls, in an ELISA based on the use of 50 ng human mitochondrial HSP60 as a captured Ag [31]. Anti-HSP60 autoAb was found in 26% of SLE patients and controls, with 500 ng/ml recombinant HSP60 as coated Ag [32]; there were 39% of SLE patients, compared with 4% of healthy controls with 0.5 μg recombinant HSP60 [6]. This frequency raises to 76% when the sera are screened by WB. Positivity in the anti-HSP60 Ab test suggests that the autoAb could contribute to the pathogenicity of SLE. This is strengthened by the observation indicating that anti-HSP60 Abs are more present in systemic AIDs associated with vasculitis than in those without vasculitis. Therefore, among AECA-positive patients with a vascular AID such as SLE, BD, PAN, WG, and microscopic polyangiitis, the anti-HSP60 frequency was of 76, 44, 79, 56, and 64%, respectively. At variance with these results, HSP60 reactivity was as low as of 20, 20, and 30%, in SS, RA, and PM, respectively, all diseases devoid of vasculitis [6]. The rarity of anti-HSP60 autoAb was confirmed in other studies of SS [31, 32] and RA [33, 34], although discrepant results have been obtained in PM [33] and RA [32]. Intriguingly, the frequency of HSP60-specific B-cell clones would be higher in the rheumatoid synovial tissue, compared with the blood B cells [35].

The expansion of the cellular immune response following activation by bacterial and/or self HSP60s [36] notwithstanding, the presence of the corresponding autoAbs indicates that such humoral response may substantially take part in the pathogenesis of vasculitis, while its contribution is negligible, if any, in nonvasculitic-associated AIDs.

Pathogenicity

HSP60

The fact that in response to a stress ECs harbor HSP60 supports the notion that it is involved in the pathogenesis of vasculitides [37]. Shedding of HSP60 and its subsequent binding to EC membrane may be critical to the pathophysiology response [6]. It has thus been observed that an increased level of soluble HSP60 parallels inflammation markers, at least in atherosclerosis [38]. It is relevant to this topic that SLE patients are at risk of developing atherosclerosis [39]. There is even an increase in their proportionate death from vascular disease, and particularly from atherosclerosis [40].

Hence, anti-HSP60 Abs might derive from cross-reactivity between invader's HSP60 and self-HSP60, or recognition of newly expressed endogenous HSP60. However, the presence of anti-HSP60 Abs has been observed in healthy individuals [41], as previously reported for AECAs [42]. This has led to the claim that they could be an early nonspecific defense against pathogens from the commensal flore [43]. Furthermore, lower level of anti-HSP60 in some AIDS implies that they may have a protective role in this development.

Anti-HSP60 Antibodies

Anti-HSP60 may be implicated in the pathogenesis of vasculitides. It has been shown that they are able to trigger cell-mediated and complement-mediated cytotoxicity at the expense of ECs [44]. Their deleterious functions may also be ascribed to their capacity to induce ECs into apoptosis [5, 6]. The latter observation has been made with AECA-positive IgG from SLE sera containing anti-HSP60 reactivity. These data turn HSP60 into an important autoAg in the AECA-triggered apoptotic process on ECs [45]. That is not a unique target as inhibitory experiments with purified IgG previously incubated with recombinant HSP60 reduce only part of apoptosis [6]. Overall, so many data substantiate the view that anti-HSP60 autoAbs contribute to the pathogenic effects of vasculitis-associated AIDs.

Although HSP60 reactivity might be associated with disease activity, immunosuppressants do not alter the level of anti-HSP60 [6], at least in PAN, WG, or SLE. Another appealing hypothesis relies on discrepant results of HSP60 reactivities. This raises the possibility that anti-HSP60 autoAb may be classified according to their specificities, and each associated with a given disease. Such diversity has been evoked for natural anti-HSP60 Ab [46]. Each of two short peptides are recognized by IgG from one donor IgG without bacterial cross-reactivity. Furthermore, it has been demonstrated in atherosclerosis that eight amino-acid sequences were differently recognized by anti-HSP60 autoAb, depending on the severity of the disease [47].

Conclusion

Despite extensive studies, specificities of AECA are scarcely identified. Interestingly, HSP60 is targeted by autoAbs in primary and in secondary vascular diseases. Based on their ability to induce EC cytotoxicity and apoptosis, it is suspected that anti-HSP60 autoAbs could play an important role in the inflammatory process of the vessel walls. However, protective functions have also been suggested in some circumstances. To conciliate both assumption, one may conceive that anti-HSP60 autoAbs comprise a heterogeneous family of Abs with different epitope specificities. Each HSP60 epitope could support the signature of one vasculitis, may characterize the disease severity, and might trigger different functional effects.

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