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Review

Anti-centromere protein A antibodies in systemic sclerosis: Significance and origin

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ABSTRACT

Systemic sclerosis (SSc) is systemic, autoimmune, connective tissue disorder characterized by vascular abnormalities, collagen deposition (fibrosis), and the production of autoantibodies to nuclear proteins. About 20%–40% of patients have antibodies to centromere protein (CENP)-A or -B. Despite the known association of anti-CENP antibodies with certain clinical features of SSc, the role of these antibodies in SSc physiopathology is still poorly understood. To better understand the clinical significance and origin of these antibodies, we and others have been studying the epitopic motifs (amino acid contact sites) on CENP-A with the aim of determining whether other proteins can prime or be targeted by them. Here, we review published and ongoing studies aimed at defining the fine specificity and origin of anti–CENP-A antibodies. We describe progress made in identifying the CENP-A epitopic motif amino acids, and the discovery of one of these motifs in forkhead box protein E3 (FOXE-3), a transcription factor previously studied only for its role in the development of lens fiber cells. Moreover, we discuss preliminary evidence for a possible role of FOXE-3 in SSc pathogenesis and for the association of different subsets of anti–CENP-A antibodies, heterogeneously expressed among SSc patients, with some clinical correlates.

Contents

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1. Introduction

Systemic sclerosis (SSc)[1] is a multisystem, autoimmune, connective tissue disorder that causes widespread fibrosis of the skin and internal organs. The clinical hallmarks of SSc are vascular abnormalities, excessive collagen synthesis, and high-titer autoantibody production to proteins of the nucleus and nucleolus [1–4]. Among systemic autoimmune disorders, SSc is one of the most disabling and invalidating diseases, severely affecting the quality of life [5,6]. Recent data have highlighted the poor efficacy of currently available drugs on skin sclerosis, interstitial lung disease, and pulmonary hypertension [7], the latter two conditions being the major causes of death in these patients. Because of the lack of effective medicines, the severity and the relatively rarity of this disease, which affects between 50 and 300 cases per million persons with an incidence ranging from 2.3 to 22.8 cases/million year [8,9], SSc is listed as an orphan by Orphanet and the European League Against Rheumatism. Very little is known about the disease's pathogenesis, starting from early inflammatory events to fibrosis of the cutis and internal organs.

Immune system activation in SSc is demonstrated by the increase of B-cell activation markers [10,11], by the ability of fibroblasts to trigger an oligoclonal T-cell response in the early stage of the disease [3,12], and by the expression of antinuclear antibodies (ANA) [2,13]. ANA are found in the sera of more than 95% of SSc patients, but their antigenic specificity varies with the clinical characteristics of the disease (Table 1) [13–84]. The most frequently targeted autoantigens are alpha-topoisomerase, in 15%–40% of patients, and the centromere proteins (CENPs) [14], mainly protein A (CENP-A) and protein B (CENP-B), in 20%–40% of patients. Anti-alpha-topoisomerase antibodies (ATA) are more often found in patients with diffuse cutaneous involvement, interstitial lung disease [85], and renal involvement [2,86]. By contrast, patients expressing anti-CENP antibodies (ACA) present a higher risk of developing pulmonary arterial hypertension and a lower risk of early interstitial lung disease in the early phase of the disease [21,27]. In addition, ACA-positive patients more often present a limited cutaneous involvement and a slower development of nailfold capillary damage [2,16,30] than do SSc patients with other ANA. In fact, registry studies [24,87] documented ACA in 80% of patients with limited cutaneous involvement but in only 10% of patients with diffuse skin involvement (reviewed in ref. [13]).

ACA and ATA are mutually exclusive in SSc, as the concomitant expression of both antibodies is extremely rare [24,30,81,86]. ACA may be found in the sera of SSc patients even years before the disease manifests clinically, and they continue to be expressed throughout the disease course [88,89]. Indeed, an elevated ACA titer is one criterion defining undifferentiated connective tissue disease at risk for SSc (early SSc) [90,91]. Patients with Raynaud's phenomenon (RP) who also express ACA are at a higher risk of developing SSc than are RP patients without ACA [85,92–94]. Finally, ACA are rarely found in other autoimmune diseases [95] or in healthy individuals [96].

All these considerations indicate that ACA are almost specific for SSc [97]. Moreover, the association of ACA with certain clinical features of SSc suggests that they have a role in the pathogenesis of this disease [98]. Here, we review our published and ongoing work on the significance and origin of ACA in these patients.

[1] **ACA**: anti-centromere-associated protein antibody; **ANA**: antinuclear antibody; **ATA**: anti-α topoisomerase antibody; **BSA**: bovine serum albumin; **CENP-A**: centromere protein A; **EMT**: epithelial to mesenchymal transition; **FGF**: fibroblast growth factor; **FOXE-3**: forkhead box protein E3; **GM-CSF**: granulocyte/monocyte-colony stimulating factor; **HD**: healthy donors; **IL-4**: interleukin-4; **IVIG**: intravenous immunoglobulin for human use; **LEC**: lens epithelial cells; **PDPL**: phage display peptide library; **PDGF**, platelet-derived growth factor; **SLE**: systemic lupus erythematosus; **SSc**: systemic sclerosis; **TGF-β**: transforming growth factor-β.

2. Pathogenicity of ACA

As the name implies, CENPs are intracellular, nuclear proteins involved in the assembly of the kinetochore during cell division [99]. CENPs specify the mitotic behavior of chromosomes, and thus are essential for mitotic progression and chromosome segregation [100]. The presence of ACA in the sera of SSc patients suggests that CENPs come in contact with the immune system so as to trigger an autoimmune response. The IgG isotype of ACA [101,102] suggests that these patients have a persistent T-cell–mediated immune response influenced by certain HLA allotypes, namely, HLA-DR1, -DR4, -DR8, and -DQB1 [103,104]. These findings are typical of most antigen-driven immune responses.

Despite these observations, the pathogenicity of ACA in SSc is a matter of debate. First, a correlation between ACA titers and SSc severity (or activity) has not yet been demonstrated and, in fact, the ACA titer appears to be relatively stable over the disease course [88,105]. In contrast, in SSc patients who express ATA, a reduction in titer is associated with a favorable outcome [106,107]. Second, so far there is no evidence that SSc can be passively transferred between animals by the administration of ACA, hence ACA do not meet one of the main experimental criteria supporting the pathogenicity of antibodies in autoimmune diseases (reviewed in ref. [108]). Third, although ACA have been demonstrated to disrupt mitosis *in vitro* [109,110], evidence is currently lacking to support any plausible pathogenetic mechanism, especially because their target antigens have a nuclear localization, rendering them inaccessible (in theory) to the immune system. It may be possible that ACA reach their antigens in the nuclei of intact cells through the transmembrane passage of immunoglobulins (reviewed in ref. [108]). Alternatively, they may interact with their antigens in apoptotic blebs according to a mechanism similar to that reported for the generation of anti-DNA antibodies in systemic lupus erythematosus (SLE) [111]. If this is the case, it is difficult to explain how ACA are generated in some patients at such high titers (>1:1280), considering that CENPs represent a minor nuclear component [112]. Finally, it is unclear why ACA associate with some SSc phenotypes but not with others.

In an attempt to explain the association of ACA with certain clinical features of SSc as well as the notably high ACA titers often detected in sera, we hypothesized that ACA cross-react with other antigens that share sequence homology with CENPs. As a first step in testing this hypothesis, we defined the fine specificity of antibodies specific to CENP-A.

3. Immunodominant epitopes of CENP-A

CENP-A, like other CENPs, belongs to the kinetochore, a centromeric protein complex essential for mitosis [100] and thus is localized at all active centromeres [99,113]. This 17-kDa protein is a minor nuclear component, representing 0.025% of all chromatin proteins in HeLa cells [112]. It is homologous to histone H3 in its central and carboxyl terminal portions, but its amino terminal portion (residues 1 to 45) is unique [112,114]. In 2006, Akbarali et al. [115] described two immunodominant epitopes of CENP-A, identified by peptide scanning analysis of sera from SSc patients with anti–CENP-A antibodies. These epitopes were localized to the amino terminus: peptide Ap1–17 spanned residues 1 to 17 (MGPRRRSRKPEAPRRRS), while peptide Ap17–30 corresponded to residues 17 to 30 (SPSPTPTPGPSRRG).

That peptides Ap1–17 and Ap17–30 represent the immunodominant epitopes targeted by anti–CENP-A antibodies was confirmed by us in a series of 75 SSc patients who screened positive for ACA on a commercially available ELISA kit: 66 (88.0%) had antibodies reacting to Ap1–17 and 62 (82.6%) were seropositive to Ap17–30, while only three (4%) were negative for both [116]. Because the antigen in the ELISA kit was recombinant CENP-B, which is not homologous to CENP-A, our findings show that most patients with ACA have antibodies to both CENPs. Additionally, by receiver operating characteristic analysis in an extended number of 85 SSc patients with ACA and 54 healthy blood donors as a comparison group, we showed that the serum titer to both peptides (chemically