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Review

Autoantibodies in systemic sclerosis

Sonal Mehra [a], Jennifer Walker [b], Karen Patterson [c], Marvin J. Fritzler [a,*]

[a] Faculty of Medicine, University of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta, Canada, T2N 4N1
[b] Department of Immunology, Allergy and Arthritis, Flinders Medical Centre, Adelaide, Australia
[c] Flinders University of South Australia, Australia

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ABSTRACT

Autoantibodies directed against a variety of nuclear, cytoplasmic and extracellular autoantigens are a serological hallmark of systemic sclerosis. This review provides an overview of the history and clinical association of many of the autoantibodies identified in SSc sera to date. Some of these autoantibodies predate the clinical diagnosis of SSc, some are pathogenic while others have no apparent role in pathogenesis. It was once thought that the autoantibody spectrum of individual SSc sera were less complex than other systemic autoimmune rheumatic diseases with respect to heterogeneous B cell responses reflected in circulating autoantibodies. However, with the advent of array technologies, there is now an unprecedented capability to detect multiple autoantibodies in an individual serum and this long held tenet of clinical diagnostic immunology is being reexamined.

Contents

Abbreviations: ANA, anti-nuclear antibody; ATA, anti-topoisomerase antibody; AMA, anti-mitochondrial antibody; AAV, ANCA associated vasculitis; AFA, anti-fibroblast antibody; AECA, anti-endothelial cell antibody; anti-CCP, anti-cyclic citrullinated peptide; anti-CENP, anti-centromere protein; dcSSc, diffuse cutaneous systemic sclerosis; ELISA, enzyme linked immunoassay; HMG, high-mobility group; IB, immunoblot; IIF, indirect immunofluorescence; LIA, line immunoassay; IP, immunoprecipitation; kDa, kiloDalton; lcSSc, limited cutaneous systemic sclerosis; MCTD, mixed connective tissue disease; MMP, matrix metalloproteinase; MPO, myeloperoxidase; PAH, pulmonary artery hypertension; PBC, primary biliary cirrhosis; PDGFR, platelet-derived growth factor receptor; PR3, proteinase 3; qPCR, quantitative primer chain reaction; RA, rheumatoid arthritis; RF, rheumatoid factor; RNP, ribonucleoprotein; RNAP, RNA polymerase; SARD, systemic autoimmune rheumatic diseases; SSc, systemic sclerosis; SLE, systemic lupus erythematosus.

* Corresponding author at: Faculty of Medicine23939, University of Calgary, 3330 Hospital Dr. NW, Calgary, AB, Canada T2N 4N1. Tel.: +1 403 220 3533.
E-mail address: fritzler@ucalgary.ca (M.J. Fritzler).

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1. Introduction and background

Systemic sclerosis (SSc) is an idiopathic systemic autoimmune rheumatic disease (SARD) characterized by severe and often progressive cutaneous and visceral fibrosis; fibroproliferative vasculopathies and pronounced cellular and humoral immunity abnormalities [1–3]. In the United States, SSc affects 1 in 4000 adults, has a female preponderance and is more frequent in African-Americans than Caucasians [2,3]. Clinically, SSc can be divided into three main groups: limited (lcSSc), diffuse (dcSSc) and sine cutaneous disease [4–6]. Classification criteria for SSc date to 1980 when the American College of Rheumatology proposed a paradigm that would distinguish SSc from other SARD [7]. These criteria have been revised to accommodate a wider spectrum of the clinical spectrum, particularly lcSSc [5,6], although this is a work that is still in progress [8].

Autoantibodies in SSc sera that target a variety of intra- and extracellular targets are a widely acknowledged hallmark of the disease [9–12]. Autoantibodies are seen at first diagnosis in more than 95% of SSc patients [11] and have been associated with distinct disease subtypes and with differences in disease severity, including extent of skin involvement, internal organ manifestations and prognosis. As reviewed later, anti-endothelial cell antibodies, anti-fibrillin-1 antibodies, anti-matrix metalloproteinases and anti-platelet-derived growth factor receptor antibodies have been associated with certain pathogenic features, providing some insight into the molecular pathogenesis of SSc and mechanisms implicated in fibrosis. There is hope that in uncovering and elucidating these processes has helped the search for potentially effective therapeutic agents. This review will focus on autoantibodies described in SSc with an emphasis on both classical and recently discovered autoantibodies and their relevance to disease phenotypes, clinical course and progression.

2. Techniques used to detect autoantibodies

At the outset, it is important to understand that the strength and validity of autoantibody associations with clinical features of SSc can vary according to the diagnostic techniques being used, but this variation is compounded by demographic, geographic, environmental and genetic factors [13,14]. With the emergence of more sensitive immunoassays, such as ELISA, addressable laser bead immunoassays (ALBIA) and chemiluminescence, care must be taken to ensure that cut-offs are based on appropriate local normal and comparative disease controls [15–17]. In addition, the source and physicochemical characteristics (i.e. recombinant vs. native, peptide vs. full length) of the autoantigen used in these newer assays must be considered because the use of recombinant antigens, especially short polypeptide epitopes [18,19], may give different results than an assay that detects reactivity to native antigens such as those represented in indirect immunofluorescence (IIF) cell based assays, immunoblotting or immunoprecipitation (IP). That being said, it is also important to recognize limitations of these assays. IIF can be negative (false negative) even when high titer antibodies to native antigens are present [20–23]. Immunoblotting almost invariably uses denaturing conditions and although some protein refolding may occur when they are transferred from polyacrylamide gels to nitrocellulose or other membranes, conformational epitopes might be underrepresented while cryptic epitopes, otherwise unavailable for binding in other assays (i.e. IIF) may now be exposed and bound to the cognate autoantibody. Similarly, IP has challenges because many SSc B cell target autoantigens are represented as heterogeneous macromolecular complexes *in vivo* and in cell extracts. Thus, typically numerous protein bands are seen in IP assays but which protein is actually targeted by the B cell can only be determined by additional analysis, such as an immunoblot of the IP proteins. A more recent approach is to IP the ribonucleoprotein macromolecular complexes from cell extracts, extract the RNA, the cDNA is then reverse transcribed from the RNA components and the RNA of interest (i.e. U3 RNA, Th RNA) detected by quantitative primer chain reaction (qPCR) using custom primers [24]. In a study of 22 anti-Th/To, 12 anti-U3RNP and 88 controls, this qPCR assay had a near perfect correlation with IP [24]. However, the technical aspects of this study suggest that it is unlikely to be adopted in high throughput diagnostic laboratories, it is only relevant to antibody targets that include RNA as a components of their macromolecular complexes and, like standard IP, the autoantibody target is inferred and not directly identified.

The bottom line is that no immunoassay is without limitations and evaluators of the medical literature that includes autoantibody testing in SSc need to be aware of these limitations. Accordingly, it is likely not realistic to declare that one assay is the gold standard by which all other assays must be evaluated. Given the technical complexities of immunoassays used to detect autoantibodies, it is understandable that, despite years of activity, standardization of autoantibody assays,

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