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Short Communication

Proteomic profiling of antigens in circulating immune complexes associated with each of seven autoimmune diseases

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ABSTRACT

Objective: Immune complexes (ICs) trigger humoral immune responses. Therefore, the identification of constituent antigens within ICs would have very different clinical significance than identification of free antigens.

Design and methods: Here, we applied immune complexome analysis of serum to the study of seven major autoimmune diseases—anti-neutrophil cytoplasmic antibody-associated vasculitis, Takayasu's arteritis, mixed connective tissue disease, dermatomyositis, Sjögren's syndrome, systemic scleroderma, and systemic lupus erythematosus—and healthy donors to comprehensively identify antigens incorporated into circulating ICs and to find disease-specific antigens.

Results: We identified 468 distinct IC-associated antigens using this method. Importantly, 62 of those antigens were disease-specific antigens, and there were at least three disease-specific antigens for each of the seven autoimmune diseases. Of the disease-specific antigens identified, coiled-coil domain-containing protein 158 and spectrin were identified as potential autoantigens important to SSc and SS pathogenesis, respectively; notable titin and spectrin autoantibodies are reportedly found in SSc and SS patients, respectively.

Conclusion: Immune complexome analysis may be generally applicable to the study of the relationship between ICs and autoimmune diseases in animals and humans.

Introduction

For a long time, immune complexes (ICs) assembly were thought to represent a common pathogenic pathway for several diseases (infections, vasculitis, and connective tissue autoimmune disorders). Actually, concentrations of circulating ICs (CICs) in sera from patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or systemic scleroderma (SSc) were significantly higher than those in sera from healthy controls [1,2]. Many researchers have investigated the mechanisms by which ICs could underlie pathogenicity [3]. An autoimmune response is directed against several autoantigens [4,5]; therefore, comprehensive profiling of the autoantigens that actually assemble into ICs may provide insight into the pathophysiology of specific autoimmune diseases, and such profiling could form the basis for novel diagnosis and treatment strategies for these diseases. However, such comprehensive profiling studies for CICs are limited because tools for screening of ICs are lacking.

We developed a proteomic strategy, designated immune complexome analysis, in which ICs are separated from whole serum and then subjected to direct tryptic digestion and nano-liquid chromatography–tandem mass spectrometry (nano-LC–MS/MS) to comprehensively identify and profile constituent antigens in CICs [6]. We used this method to identify CIC-associated antigens in sera from patients with RA, and found that thrombospondin-1 is a constituent of CICs and is more highly specific and sensitive for established and early RA than other conventional diagnostic markers such as rheumatoid factor or anti-citrulline-containing protein/peptide antibody [6,7].

In this report, we used immune complexome analysis of serum to study seven major autoimmune diseases—anti-neutrophil cytoplasmic antibody-associated vasculitis (AAV), Takayasu's arteritis (TA), mixed connective tissue disease (MCTD), dermatomyositis (DM), Sjögren's syndrome (SS), SSc, and SLE—to comprehensively identify antigens incorporated into CICs and find disease-specific antigens.

Abbreviations: AAV, anti-neutrophil cytoplasmic antibody-associated vasculitis; CICs, circulating immune complexes; DM, dermatomyositis; ICs, immune complexes; MCTD, mixed connective tissue disease; nano-LC–MS/MS, nano-liquid chromatography–tandem mass spectrometry; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; SSc, systemic scleroderma; TA, Takayasu's arteritis

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Table 1
Summary of disease-specific antigens in CICs isolated from patients with AAV, TA, MCTD, DM, SS, SSc, or SLE.

Accession	Description	AAV (n = 7) Frequency/peptides per hit	TA (n = 7) Frequency/peptides per hit	MCTD (n = 9) Frequency/peptides per hit	DM (n = 8) Frequency/peptides per hit	SS (n = 14) Frequency/peptides per hit	SSc (n = 7) Frequency/peptides per hit	SLE (n = 14) Frequency/peptides per hit
Protein G								
IPI00926948.1	Uncharacterized protein	3/1						
IPI00217511.1	Seven transmembrane helix receptor	2/1						
IPI00555948.1	Androgen-regulated short-chain dehydrogenase/reductase 1 variant (fragment)			2/1				
IPI01012262.1	Uncharacterized protein			2/1				
IPI00910896.1	cDNA FLJ56556, highly similar to alpha-1-syntrophin				2/1			
IPI00982295.1	Uncharacterized protein				2/1			
IPI00023711.2	Envoplakin					2/1		
IPI00103630.3	Isoform 2 of protein phosphatase 1E					3/1		
IPI00249982.4	Isoform 1 of death-inducer obliterator 1					2/1–9		
IPI00643437.1	Uncharacterized protein					3/1		
IPI00784980.1	Isoform 1 of coiled-coil domain-containing protein 158					2/1–3		
IPI00796214.1	Isoform 2 of mediator of RNA polymerase II transcription subunit 12-like protein					2/1		
IPI00796249.1	Uncharacterized protein					4/1		
IPI00796316.5	Uncharacterized protein					7/1–3		
IPI00829812.3	13 kDa protein					2/1		
IPI00844250.2	cDNA FLJ52101					2/1		
IPI00853581.1	Uncharacterized protein					3/1–2		
IPI00917789.1	Uncharacterized protein					2/2		
IPI00979799.1	Similar to VH-7 family (N54P3)D/J protein					2/2		
IPI00968154.1	Uncharacterized protein						2/1	
IPI00946590.1	26 kDa protein						2/1	
IPI00447173.1	Antigen MLAA-39						2/1	
IPI00973260.1	cDNA FLJ55558, highly similar to regulating synaptic membrane exocytosis protein 1						2/1	
IPI01009738.1	Isoform 3 of Golgin subfamily A member 4						2/1	
IPI00436634.4	Isoform 3 of nipped-B-like protein						2/1	
IPI00006900.1	Something about silencing protein 10						2/1	
IPI00304557.2	Short palate, lung and nasal epithelium carcinoma-associated protein 2							3/1
IPI00827548.3	cDNA FLJ44825 fis, clone BRACE3046609, highly similar to *Homo sapiens* inhibitor of Bruton agammaglobulinemia tyrosine kinase (IBTK), mRNA							2/1