In-Depth Serum Proteomics by DIA-MS with In Silico Spectral Libraries Reveals Dynamics during the Active Phase of Systemic Juvenile Idiopathic Arthritis

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ABSTRACT: In serum proteomics using mass spectrometry, the number of detectable proteins is reduced due to high-abundance proteins, such as albumin. However, recently developed data-independent acquisition mass spectrometry (DIA-MS) proteomics technology has made it possible to remarkably improve the number of proteins in a serum analysis by removing high-abundance proteins. Using this technology, we analyzed sera from patients with systemic juvenile idiopathic arthritis (sJIA), a rare pediatric disease. As a result, we identified 2727 proteins with a wide dynamic range derived from various tissue leakages. We also selected 591 proteins that differed significantly in their active phases. These proteins were involved in many inflammatory processes, and we also identified immunoproteasomes, which were not previously found in serum, suggesting that they may be involved in the pathogenesis of sJIA. A detailed high-depth DIA-MS proteomic analysis of serum may be useful for understanding the pathogenesis of sJIA and may provide clues for the development of new biomarkers.

INTRODUCTION

In recent years, the proteome analysis of clinical samples using mass spectrometry (MS) has been attracting attention as a powerful tool for elucidating pathological conditions, identifying disease-related proteins as candidate biomarkers, and searching for drug targets. In particular, serum represents an important clinical sample that can be collected in a minimally invasive manner, which is widely used in clinical practice for biochemical tests and biomarker measurements. The advantages of serum are its simplicity of collection and ease of storage.

Serum protein concentrations are known to have a wide dynamic range. In an MS-based proteome analysis, the number of detectable proteins is limited by the effect of high-abundance proteins such as albumin and γ-globulin. To reduce the dynamic range of these protein concentrations, high-abundance protein depletion methods using antibody columns are actively used. However, a conventional data-dependent acquisition mass spectrometry (DDA-MS) based single-shot proteome analysis does not provide sufficient proteome coverage, even for depleted serum. Because of this difficulty, a SOMAscan assay using aptamers and a multiplex assay using an antibody-based proximity extension assay have recently attracted attention as methods for identifying more than 1000 proteins from serum without using MS. However, MS-based proteome analysis techniques have also undergone great technological innovation. The advent of data-independent acquisition mass spectrometry (DIA-MS) has dramatically expanded the proteome coverage of a single-shot proteome analysis. Furthermore, the performance of DIA-MS has been dramatically improved by the development of overlapping window DIA-MS, which enables the detection of small amounts of proteins and algorithms that can enable spectral libraries to be generated from sequence information using deep learning. Combining the high-abundance protein removal method in serum and advanced DIA-MS technology makes it possible to detect a large amount of proteins by a single-shot proteome analysis. Therefore, it is also expected to identify previous unknown factors and pathologies related to diseases and novel biomarker proteins.

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Systemic juvenile idiopathic arthritis (sJIA) is a rare autoinflammatory disease that occurs in children. sJIA is chronic arthritis that causes systemic symptoms, such as high spiking fever, salmon-pink rash, lymphadenopathy, hepatosplenomegaly, and serositis. Macrophage activation syndrome (MAS), a secondary hemophagocytic lymphohistiocytosis (HLH), complicates about 10% of patients with sJIA. As MAS causes rapid progression of life-threatening conditions with a mortality rate of up to 17% and requires accurate and prompt evaluations and treatment, biomarkers for assessing MAS are also required. Interleukin-18 (IL-18) and soluble CD163 have been reported to be valuable biomarkers for distinguishing sJIA from other diseases. C-X-C motif chemokine ligand 9 (CXCL9) and adenosine deaminase 2 (ADA2) have been reported to be useful biomarkers denoting the active phase with MAS.

The overproduction of cytokines, such as IL-6, IL-18, and IL-1β, is the leading cause of inflammation in sJIA. It is also known that the driving cytokines, including TNFα and IFNγ, are altered during MAS in sJIA patients. However, the pathogenesis leading to systemic inflammation and the mechanisms underlying the prolonged inflammation remain unclear. Recently, it was reported that the analysis of sJIA patient serum using SOMAselect identified a new candidate biomarker and variable protein groups in sJIA that were associated with pulmonary alveolar proteinosis (PAP). Thus, detailed studies of changes in serum protein levels using proteome analysis techniques may reveal important factors related to the disease and proteins that can serve as new biomarkers, which may provide clues to understanding the pathogenesis of the disease.
Figure 2. Changes in protein profiling during sJIA activity. (A) Volcano plot showing the expression in the active w/MAS and inactive phases with fold changes and p-values. The line on the horizontal axis represents $p = 0.05$. (B) Volcano plot showing the expression in the active w/oMAS and inactive phases with fold changes and p-values. The line on the horizontal axis represents $p = 0.05$. (C) Venn diagram with the significant proteins obtained in panels (A) and (B). (D) Scatter plot with the abundant difference ratio of significant proteins in active phases w/MAS and w/oMAS. (E) Heat map shows the protein groups that were significantly differentially abundant. The log-transformed protein quantitative values were standardized by z-score and color-coded.
In this study, we performed a proteomic analysis of sJIA serum by overlapping window DIA-MS to evaluate the performance of the analytical approach and reveal changes in proteome profiling according to disease activity. We also investigated the comparison with previously reported biomarkers and biological functions that might be associated with the pathogenesis of sJIA using a group of differentially abundant proteins.

**RESULTS AND DISCUSSION**

**Evaluation of MS Analytical Performance in sJIA Serum.** Our MS analysis identified 2727 proteins using all samples. From these proteins, we searched for previously reported sJIA biomarkers, inflammatory-related proteins, and high-abundance proteins in each tissue. The latter group of organ-specific protein sets was examined using a set of genes provided by the HPA that showed elevated expression levels in comparison to other tissues. We ranked the standardized MS data based on abundance and colored the proteins according to protein groups (Figure 1A,B). As expected, inflammatory proteins and sJIA-associated biomarker proteins, excluding low-abundance substances (e.g., IL-6 and CXCL9), were detected in the high abundance range. On the other hand, some low-abundance proteins, such as cytokines and chemokines, were also detected (Figure 1A). Notably, we found many proteins over a wide range of concentrations, not only many high-abundance proteins produced in the liver but also tissue leakage proteins produced in the lungs and intestines (Figure 1B). These results confirmed that our analysis system can be established as a single-shot proteome analysis with a wide dynamic range. Next, we compared the measurement intensities of the serum proteome analysis with the laboratory measurements (IL-18 was measured by enzyme-linked immunosorbent assay, ELISA). As shown in Figure 1C, CRP, ferritin, and IL-18 showed a high correlation in all cases. Therefore, our serum proteome analysis system may be suitable for a serum protein profiling analysis and biomarker discovery because of its wide analytical depth and comparative quantitative analysis.

In this analysis, to accommodate the wide dynamic range of serum protein concentrations, the width of the isolation windows for DIA-MS was narrowed to 4 m/z to limit the types of ionized peptides that could pass the quadrupole, and the accumulation time of C-trap for fragmented ions was increased (MS2 accumulation time: 77 ms). In addition, by measuring with the overlapping window DIA, MS/MS spectra in the overlapping isolation windows were computationally demultiplexed to reduce the complexity of MS/MS spectra. This enabled a proteome analysis in a wide dynamic range. However, the number of scans in the DIA-MS method had to be reduced because the narrow isolation window width and long C-trap accumulation time increased the cycle time of the MS analysis. We reduced the number of scans by limiting the scan window range to 500–740 m/z, where peptides were richly detected. This DIA-MS method reduced the sequence coverage by narrowing the scan window range but was able to detect minor peaks in the 500–740 m/z range, thereby expanding the proteome coverage. The fact that we were able to detect more than 2,700 proteins in the serum showed the power of this DIA-MS method.

**sJIA Sera Proteomics Profiling.** We analyzed the changes in serum proteins according to the different stages of sJIA, which required a detailed quantitative analysis. We analyzed a total of 2032 proteins after data cleaning for all identified proteins. These identified protein expression tables are shown in Table S1.

First, we compared proteins whose abundance changed between active (w/MAS and w/oMAS) and inactive phases: 1153 proteins (56.7%) were identified as statistically significant proteins during the active phase w/MAS and 666 proteins (32.8%) were identified as statistically significant proteins during the active phase w/oMAS (Figure 2A,B). Significantly upregulated proteins included many inflammatory proteins (e.g., CRP, ferritin [ferritin light chain; FTL, heavy chain; FTH1], and serum amyloid A proteins [SAA-1, SAA-2]). We also detected proteins, such as soluble CD163 and IL-18, that had previously been reported to be elevated during the active phase.

Among these significant protein groups, 591 proteins were common and 562 were significant only during the active phase w/MAS (Figure 2C). Approximately 90% of the proteins that changed in the active phase w/oMAS were included in the active phase w/MAS proteins.

Next, we evaluated the difference in the abundance of the common 591 proteins (Table S2). The changes in protein abundance at each phase were positively correlated, and it was revealed that this protein group showed the same pattern of change, regardless of the presence or absence of MAS (Figure 2D). A hierarchical clustering analysis showed that these protein intensity changes were stratified by phase and tended to be enhanced by the complication of MAS (Figure 2E). The major proteins that were strongly upregulated in the active phase (w/MAS and w/oMAS) were SAA-2, FTL, FTH1, leucine aminopeptidase 3 (LAP3), heat shock protein (HSP90AA2P), and proteasome subunit (PSMB10). Proteins for which the expression was strongly downregulated were insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein 5 (IGFBP5) (Figure 2D). These proteins also showed significant differences in the comparative analysis of each stage of the disease and may be proteins that reflect the disease activity (Figure S1).

In this analysis, we successfully identified a large set of 2727 proteins with a high quantitative performance from sJIA patient serum using a high-depth DIA-MS proteomic analysis. In recent sJIA serum proteomics studies using SOMAscan, a total of 1317 protein sets were used for the analysis. In comparison to the total proteins obtained in our analysis, 570 proteins overlapped, but many unique proteins were also obtained in each of the analyses (Figure S2A). Focusing on the proteins that changed during the active phase of sJIA, 55 proteins were found in common, despite differences in the comorbidities and race of the patients studied (Figure S2B and Table S3). SOMAscan is a method for detecting proteins by aptamer-based assays. Although this method only targets proteins that can be detected in a predetermined set, it allows the identification of low-abundance proteins, such as cytokines and chemokines, which are outside the dynamic range measurable by mass spectrometry. This feature makes it highly useful for the identification of a wide range of biomarkers. On the other hand, the DIA-MS proteomics that we used in this study is a nontargeted analysis that matches an extensive database of human proteins. Many proteins can be detected depending on the condition of the sample.
be useful for the pathological analysis of various diseases and the development of serum biomarkers that use them for different purposes or combine them.

**Functional and Network Analysis of Differentially Abundant Proteins.** To understand the functional network to which a group of proteins with significantly altered expression levels belonged, we performed an enrichment analysis of GO biological processes and KEGG pathways.

First, among the shared highly abundant proteins, many GO terms and KEGG pathways were associated with the regulation of the immune response (e.g., response to cytokine including the IL-1-mediated signaling pathway [GO:0070498, GO:0019221], T-cell receptor signaling pathway [GO:0050852], and activation of the immune response [GO:0002253] were significantly enriched) (Figures 3A and S3A). Other terms related to platelet activity (hsa04611) and neutrophil activity (GO:0043312, GO:0045321) were also highly enriched. Notably, the function of the proteasome (hsa03050, GO:0043161) was enriched in the top terms ($p = 1.2 \times 10^{-15}$), and these associated terms were identified as connecting roles for immune-related terms. Protein–protein interaction (PPI) network analysis also showed that proteasome subunit proteins interacted with many proteins associated with the regulation of inflammatory responses and neutrophil activity (Figure 4A). The components of these proteasome subunits were coregulated across the patients.

Figure 3. Network diagram of Gene Ontology (GO) and KEGG pathways by ClueGO. The same color nodes indicate similar functions. (A) Network diagram composed of upregulated proteins. (B) Network diagram composed of downregulated proteins.
Figure 4. Results of a STRING-based interaction analysis of identified proteins. This schematic diagram represents the proteins and the functional groups of the proteins. Differentially abundant proteins were classified according to Gene Ontology and KEGG pathways, and significant proteins were selected. The lines indicate the confidence level of the predicted interactions (confidence score ≥ 0.7). The color of the node represents each term, and the size represents the fold change between the active phase w/MAS and the inactive phase. The node size becomes larger with a higher fold change for upregulated proteins (A) and with a lower fold change for downregulated proteins (B). (A) Representative PPI using proteins that were all associated with the immune system process (GO:0002376), which were upregulated. (B) Representative PPI using downregulated proteins.
In particular, proteasome subunit β types 8, 9, and 10 (PSMB8, 9, and 10), which are immunoproteasome subunits, were dramatically upregulated during the active phase. This result suggests that the immunoproteasome plays an important role in biological processes and is involved in the pathogenesis of sJIA.

The group of downregulated proteins was enriched with many terms related to cholesterol metabolism (hsa04979, GO:0008203) and blood coagulation (GO:0007596) (Figures 3B and S3B). In addition, the PPI analysis revealed a complex network of proteins involved in angiogenesis (GO:0001525) and the regulation of growth (GO:0040008), which reflects the inflammation-induced outcomes (Figure 4B). In particular, IGF-1, the protein in the growth factor pathway that was most strongly downregulated during the active phase, is also associated with cell proliferation and the regulation of inflammatory cytokine gene transcription.34,35 Some reports showed that serum IL-6 in sJIA patients was inversely correlated with IGF-1 levels.36−38 The suppression of these functions may represent prolonged cytokine release and inflammation, and combination with upregulated molecules may be useful for monitoring disease activity.

Finally, we analyzed the proteins that were significantly changed only during MAS (Figure S4A). The 212 highly expressed proteins showed high enrichment in terms related to amino acid metabolism (GO:0006520) and macrophage activity (GO:0042116). Downregulated proteins included many terms related to blood coagulation (GO:0007598), angiogenesis (GO:0001525), and the response to growth factor (GO:0070848) (Figure S4B). These terms were implicated in reflecting the intensity of inflammation in the active phase w/MAS.

An important finding of the present analysis is that immunoproteasomes are identified in peripheral blood serum, reflecting the intensity of disease activity. Proteasomes are protein complexes that function as a ubiquitin–proteasome system and are involved in not only breaking down dead proteins but also many other essential cellular processes, such as cell cycle regulation and proliferation.39 Proteasomes are located in not only the cytoplasm but also the serum as circulating proteasomes (c-proteasomes). Although the exact function of c-proteasomes remains unclear, the c-proteasome levels are increased in various diseases, such as autoimmune disease, malignancy, and sepsis.40,41

An immunoproteasome is a special type of proteasome in which constitutive proteasome subunits (PSMB5, 6, 7) are converted to immunoproteasome subunits (PSMB8; 9, 10) upon stimulation with inflammatory cytokines, such as interferon-γ.42−44 Immune cells contain these immunoproteasomes, which are key regulators of immune cell activation and differentiation.45 In particular, they play a major role in inflammatory signaling by regulating the activation of inflammatory transcription factors, such as NF-κB.45,46 The proteasome identified in this study was also composed of immunoproteasome subunits, and their functional analysis showed that they formed a strong network with immune-related functions (e.g., inflammatory cytokines, T-cell activity, and NF-κB signaling).

Recently, the specific inhibition of immunoproteasomes has been shown to suppress the secretion of proinflammatory cytokines (IL-1β, IL-6, IFNγ, TNFα) and activate the expression of IL-10, providing insight into the function of the immunoproteasome in autoimmunity.47−49 In addition, Ilona Kammerl et al. found the activation of immunoproteasomes from peripheral blood mononuclear cells (PBMCs) of patients with severe COPD.50 Furthermore, because of the activation of immunoproteasomes in PBMCs stimulated by proinflammatory cytokines and because of immunoproteasome inhibition, experimental studies demonstrated cytokine suppression; they suggested that therapeutic targeting of the immunoproteasome may be a novel concept for COPD treatment. Indeed, immunoproteasome inhibitors have been clinically tested in the treatment of autoimmune polymyositis and lupus nephritis (www.clinicaltrials.gov)51−52 and are expected to be applied in the treatment of various immune diseases in the clinical setting.

Our results suggest that the activation of inflammation in sJIA may also have an important role in the immune proteasome. Although the number of cases was relatively small, the proteasome levels in the inactive samples that we analyzed were higher in patients immediately after treatment (within 2 weeks of the active phase) in comparison to patients in other inactive phases (Figure S5B). Therefore, immunoproteasome levels in the peripheral blood may be a useful biomarker that can sensitively reflect the disease activity of sJIA and enable the detailed evaluation of the treatment response.

In this study, the number of patients was small, so some proteins and functions that we found may have been affected by patient characteristics, such as age, race, and treatment. In addition, we could not evaluate disease specificity in this study. Therefore, the verification of the findings in a large cohort is necessary for the future. However, the present study demonstrates that protein profiling and the functional analysis of rare diseases may provide clues to elucidate their pathogenesis and the development of new biomarkers.

**CONCLUSIONS**

We identified a large number of proteins by pretreatment to remove high-abundance proteins from serum and a proteomics analysis using advanced DIA-MS technology. These proteins included many tissue leakage proteins, which were superior for the quantitative analysis. We also obtained a lot of information about the molecules that were differentially expressed during the active phase of sJIA and their functions. Although the clinical manifestations and key cytokines differed between the active phase w/MAS and w/oMAS samples, many proteins have a common spectrum. In addition, the immunoproteasome in peripheral blood serum identified in this study showed an increased protein expression level during the active phase of the disease, and this may provide clues to the mechanism of the disease and the development of new biomarkers. Detailed molecular profiling and analyses using proteomics may be helpful for improving the understanding of the pathogenesis of rare diseases such as sJIA.

**MATERIALS AND METHODS**

**Study Design.** We performed a cross-sectional study of patients treated for sJIA. The study was performed according to the principles of the Declaration of Helsinki and approved by the ethics review board of Chiba Children’s Hospital, Chiba, Japan (approval number, 2020-022). Written informed consent was obtained from the study participants and/or their guardians.

**Setting and Participants.** We recruited patients with sJIA at Chiba Children’s Hospital in October 2020. The eligible
participants were patients who had previously been diagnosed with sJIA based on the International League of Associations for Rheumatology (ILAR) classification criteria and who had undergone treatment at Chiba Children’s Hospital between April 2013 and March 2020. The criteria for active phase sJIA were typical symptoms, including fever, arthritis, hepatosplenomegaly, rash, and generalized lymphadenopathy, and increased CRP levels (>0.3 mg/dL). The diagnosis of MAS was based on the 2016 EULAR/ACR/PRINTO classification criteria. The exclusion criteria were missing medical records, complications of other rheumatic diseases, complications of acute infection at the time of serum collection, or other conditions that may induce inflammation, such as surgery, injury, and malignancy.

We collected sera with different disease activity (active phase with MAS [active w/MAS], n = 6; active phase without MAS [active w/oMAS], n = 5; and inactive phase, n = 13) from sJIA patients. The patients’ characteristics and laboratory data at the time of serum collection are shown in Table S4. Some patients had few or mild joint symptoms at the onset of sJIA, and their arthritis was confirmed later. The data collected from each patient did not differ substantially from the previously reported information. Therefore, the grouping of the data by disease phase was deemed reasonable. The age was consistent with the general age of sJIA patients, and the ferritin and IL-18 levels were typical symptoms, including fever, arthritis, hepatosplenomegaly, rash, and generalized lymphadenopathy, and increased CRP levels (>0.3 mg/dL). The diagnosis of MAS was based on the 2016 EULAR/ACR/PRINTO classification criteria. The exclusion criteria were missing medical records, complications of other rheumatic diseases, complications of acute infection at the time of serum collection, or other conditions that may induce inflammation, such as surgery, injury, and malignancy.

**Sample Preparation for the Proteome Analysis.** Highly abundant serum proteins were depleted from 10 μL of serum using a High Select Top 14 Abundant Protein Depletion Mini Spin Column (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer’s instructions. Depleted serum (50 μL) was diluted with 150 μL of 100 mM Tris-HCl, pH 8.5, and 2% sodium dodecyl sulfate (SDS) and treated with 10 mM dithiothreitol at 50 °C for 30 min. The sample was then alkylated with 30 mM iodoacetamide in the dark at room temperature for 30 min and subjected to cleanup.

**Proteome Analysis.** Peptides were directly injected onto a 75 μm × 40 cm PicoFrit emitter (New Objective, Woburn, MA) packed in-house with C18 core–shell particles (CAPCELL CORE MP 2.7 μm, 160 Å material; Osaka Soda Co., Ltd., Osaka, Japan) at 60 °C and then separated with a 120 min gradient at 100 mL/min using an UltiMate 3000 RSLCnano LC system (Thermo Fisher Scientific, Waltham, MA). Peptides eluted from the column were analyzed on a Q Exactive HF-X (Thermo Fisher Scientific) for overlapping window data-independent acquisition (DIA)-MS. MS1 spectra were collected in the range of 495–745 m/z at 30 000 resolutions to set an automatic gain control target of 3 × 106 ions and a maximum injection time of 55 ms. MS2 spectra were collected at >200 m/z at 45 000 resolution to set an automatic gain control target of 3 × 106 ions, a maximum injection time of ‘auto’, and stepped normalized collision energy of 22, 26, and 30%. The isolation width for MS2 was set to 4 m/z, and overlapping window patterns of 500–740 m/z were used for window placements optimized by Skyline (Table S5).

The MS files were searched against a human spectral library using Scaffold DIA (Proteome Software, Inc., Portland, OR). The predicted human spectral library was generated from the human protein sequence database (id UP000005640) established by Prosim (https://www.proteomicsdb.org/prosim/). The Scaffold DIA search parameters were as follows: experimental data search enzyme, trypsin; maximum missed cleavage sites, 1; precursor mass tolerance, 8 ppm; fragment mass tolerance, 8 ppm; and static modification, cysteine carboxamidomethylation. The protein identification threshold was set at <1% for both peptide and protein false-discovery rates. The peptide quantification was calculated by the EncyclopeDIA algorithm in Scaffold DIA. For each peptide, the four highest-quality fragment ions were selected for quantitation. The protein quantitative value was estimated from the summed peptide quantitative values. The protein values were normalized via linear median normalization between samples in the Microsoft Office 365 Excel software program (Microsoft, Redmond, WA).

**Enzyme-Linked Immunosorbent Assay (ELISA).** Serum IL-6 and IL-18 levels were measured using a commercial ELISA (IL-18: MBL, Nagoya, Japan; IL-6: R&D Systems, Inc., Minneapolis, MN), according to the manufacturer’s instructions.

**Statistical and Bioinformatics Analyses of the Data.** We performed statistical analysis and generated hierarchical clustering and heat maps using the JMP Pro 13 (SAS Institute Inc., Cary, NC) and Perseus (version 1.6.15.0; available online: https://maxquant.net/perseus/) software programs. After conducting log2 transformation for the quantified values, we used proteins with at least three peptide counts that were detected in at least one subject for the subsequent analysis, and valid values were filtered using proteins with a minimum of 70% quantified values in at least one phase. Missing values of the proteins were imputed based on a normal distribution (width = 0.5, downshift = 1.8) to simulate signals of low-abundance proteins. Significant differences between the sJIA groups were addressed by a two-tailed Student’s t-test. The p-values were corrected using the Benjamini & Hochberg procedure as post hoc tests to reduce the incidence of type I errors and false positives in the results of multiple comparison tests. Proteins with p-values of <0.05 were considered...
statistically significant. Differentially abundant proteins were subjected to z-normalization followed by hierarchical clustering with Pearson’s correlation coefficient. We analyzed the specific abundant proteins in serum using the Kruskal–Wallis test, followed by the Dunn’s multiple comparison test, to determine statistical significance.

Functional gene ontology (GO) and a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using the Enrichr online tool (http://amp.pharm.mssm.edu/Enrichr/).65 The gene set libraries from Enrichr that were referenced in each analysis were as follows: “GO_Biological_Process_2021” (terms, 6,036; gene coverage, 14,937) and “KEGG_2021_Human” (terms, 320; gene coverage, 8,078). Then, enrichment in GO terms from the biological processes and KEGG pathways for the most relevant coverage, 8,078). Then, enrichment in GO terms from the biological processes and KEGG pathways for the most relevant predicted genes was obtained using ClueGO plug-in.63 of STRING v11; http://string-db.org/) database with the highest confidence interaction score.65 The crucial proteins from the present results were referred to the Human Protein Atlas (HPA; https://www.proteinatlas.org/).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c06681.

Our data and the previously reported SOMAscan data show that 55 proteins overlap in the sJIA active phase (Table S3); clinical characteristics of the participants (Table S4); comparison between groups of major proteins with high variability in the active phase (Figure S1); comparison of the number of proteins with SOMAscan (Figure S2); enrichment analysis of gene ontology (biological process) and KEGG pathways (Figure S3); analysis of 562 proteins that only showed significant differences in active w/MAS (Figure S4); and changes in the expression of proteasome subunits (Figure S5).66

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Author Contributions

H.S. and Y.I. designed the study. H.S., Y.I., D.K., K.M., T.Y., A.Y., M.T., and A.H. treated patients and also recruited them for the study. H.S., Y.K., D.N., R.N. performed the experimental work. H.S., M.I., R.K., and Y.K. analyzed and interpreted the data. H.S., Y.K., and Y.I. wrote the manuscript. O.O. supervised the interpretation of the data. O.O. and N.S. provided guidance and made important contributions to the writing of the manuscript. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest. The mass spectrometry proteomics data have been deposited in the ProteomeXchange Consortium via the jPOST partner repository with the dataset identifier PXD030413 for ProteomeXchange and JPST001419 for jPOST.

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**ABBREVIATIONS**

ADAA2, adenosine deaminase 2; CD163, scavenger receptor cysteine-rich type 1 protein M130; CRP, C-reactive protein; CXCL9, C-X-C motif chemokine ligand 9; DDA, data dependent acquisition; DIA, data independent acquisition; DTA, data targeted acquisition; DIA, data dependent acquisition; DIA, data independent acquisition; FTH, ferritin heavy chain; FTL, ferritin light chain; GO, gene ontology; HPA, Human Protein Atlas; IFNγ, interferon γ; IGF-1, insulin-like growth factor 1; IGFBP5, insulin-like growth factor binding protein 5; IL-1β, interleukin-1β; interleukin-1βeta; IL-6, interleukin-6; LAP3, leucine aminopeptidase 3; MAS, macrophage activation syndrome; MS, mass participation.

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