Revisiting the INSPIRE trial: Antibody profiling reveals high prevalence of occult autoimmunity

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Abstract

Rationale: The INSPIRE trial failed to demonstrate improved survival in participants labeled as Idiopathic Pulmonary Fibrosis (IPF) after treatment with IFN-gamma-1β. This outcome became the impetus to develop more personalized approaches to the diagnosis, classification, and management of pulmonary fibrosis.

Objective: The present study was designed to assess autoantibody profiles in a randomly selected group of INSPIRE trial participants in order to better define IPF on a molecular diagnostic level and define subsets with potentially different underlying disease processes.

Methods: We performed conventional, gel-based protein and RNA immunoprecipitation (IP) on 483 plasma specimens derived from patients enrolled in both the treatment and placebo arms of INSPIRE. Tandem immunoprecipitation and mass spectrometry proteomics (IP-to-MS) of selected specimens was performed to confirm conventional IP interpretation and to identify unknown autoantigens.

Results: Based on conventional IP approaches, approximately 30% of trial participants had evidence of autoimmune disease-specific autoantibodies and an additional ~ 10% had evidence of autoantibodies of unknown specificity. IP-to-MS revealed additional autoantigens, including Annexin 11.

Conclusions: IP analyses demonstrated an unexpectedly high prevalence of autoantibodies potentially indicative of underlying connective tissue disease-associated ILD, underscoring the importance of classification schemes incorporating unbiased autoantibody profiling.

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Introduction

In 2009, publication of negative results from the INSPIRE (INternational study of Survival outcomes in idiopathic Pulmonary fibrosis (IPF) with InteRfEron gamma-1β) phase 3 randomized, double-blind, placebo-controlled clinical trial encompassing 826 patients labeled as “IPF” (1) generated considerable disappointment, particularly given the limited treatment options for this relentlessly progressive disease. Participants in INSPIRE were 40–79 years of age, with IPF diagnosed according to clinical, radiological, and histological criteria that have changed with the publication of four consensus statements since that time (2). The primary endpoint of INSPIRE was overall survival time, but the trial was terminated in early 2007 after a planned interim analysis showed no survival benefit from IFN gamma-1β treatment. The authors of the paper summarizing the INSPIRE trial's unfavorable findings pointed out that those with quickly progressing illnesses might have been excluded by the eligibility requirements. They proposed that selection bias favoring enrollment of patients with more stable disease may have contributed to the reported lack of efficacy of IFN gamma-1β, decreasing the chance of observing a therapeutic benefit. Another key issue potentially contributing to the failure of this trial was the heterogeneity of the study population, as patients were not rigorously screened for abnormal serology (e.g., ANA positivity) or other

clinical features of occult autoimmunity that might have skewed clinical outcomes. In fact, these considerations have prompted the search for novel biomarkers that can more accurately describe such patients and, eventually, create more individualized methods for their diagnosis and treatment (3).

The present study was therefore designed to revisit a randomly selected group of participants in the INSPIRE trial in order to define patient subsets with alternative disease processes that could potentially explain differing responses to therapy. In fact, as early as 2012 (4), the available literature highlighted subsets of patients previously diagnosed with IPF who manifested a more favorable clinical course and/or had subtle clinical features of autoimmune disease corresponding to the presence of disease-specific autoantibodies (e.g., myositis- or scleroderma-associated autoantibodies) (5) that were not assessed prior to enrollment in INSPIRE. Because the biologic behavior of interstitial lung disease and the response to immunomodulatory therapy may be quite different in the setting of underlying autoimmunity–even when associated with IPF-like histopathologic abnormalities of UIP (6, 7)–identification of these individuals is critical for appropriate placement in clinical trials and development of targeted therapeutic modalities.

Given the unique opportunity provided by the INSPIRE trial to assess the frequency of disease-specific autoantibodies in a large population of “idiopathic” ILD patients, we sought to serologically characterize these individuals based on the hypothesis that a subset of individuals previously classified as IPF would possess autoimmune disease-specific antibodies targeting aminoacyl-tRNA synthetases (Jo-1, PL-7, PL-12, EJ, OJ, KS) or scleroderma-associated antigens (including Scl-70, Th/To, U3RNP, and Pm/Scl) indicative of an underlying autoimmune diathesis. We therefore performed a combination of protein and RNA immunoprecipitation on 483 plasma specimens derived from patients enrolled in both the treatment and placebo arms of this trial. Collectively, these studies demonstrated that a substantial percentage of patients (~ 40%) classified as IPF possessed serologic markers of occult autoimmunity (e.g., myositis- or scleroderma-associated antibodies)—many of which were confirmed by IP-to-MS, a quantitative, mass spectrometry-based method of autoantibody detection. These results demonstrate the power and versatility of newer mass spectrometry-based methodologies that can more accurately identify markers of occult myositis- and scleroderma-associated ILD as well as autoantibodies more uniquely linked to IPF.

Methods

Cohort. Based on availability, plasma samples collected from 483 consented patients enrolled in the INSPIRE trial were assessed in the current study. Baseline demographic characteristics included age, sex, and disease duration. Response to treatment (IFN-gamma-1β) versus placebo was assessed through change in pulmonary function tests (absolute and percent predicted FVC), days to respiratory-related hospitalization, and survival. Banked samples from healthy subjects without interstitial lung disease were used as controls for immunoprecipitation assays. See online data supplement for additional details.

Protein immunoprecipitation. 50 µl of plasma were incubated overnight with Protein A sepharose beads, following which IgG-bound beads were co-incubated with ^{35}S-methionine labeled K562 extract* for 2 hours according to established protocol (8). Immunoprecipitated proteins were then eluted with SDS elution buffer and subjected to SDS-PAGE (Polyacrylamide Gel Electrophoresis) to delineate protein bands recognized by patient sera. Comparison to established reference sera allowed identification of specific autoantigen targets.

*K562 extract is derived from an erythroleukemia cell line that is commonly used to detect ubiquitously expressed target antigens in immunoprecipitation assays based on favorable growth characteristics and high expression levels of autoantigens targeted in a variety of systemic autoimmune diseases.

RNA immunoprecipitation. IgG-bound beads generated as above and resuspended in NET-2 buffer (50mM Tris/HCl, pH 7.4, 150 mM NaCl, 0.05% Igepal CA630) were incubated with K562 whole cell extract for 2 hours at 4°C. After washing with NET-2 buffer, the resulting complexes were resuspended in extraction buffer (NET-2, 0.25 M sodium acetate, 0.83% SDS, 1 µl glycogen) prior to phenol/chloroform/isoamyl alcohol (50:50:1) extraction. Subsequent ethanol precipitation of RNA and electrophoretic resolution on a 7 M urea 8% polyacrylamide gel permitted visualization through neutral silver staining. Comparison of electrophoretic mobility with controls of known specificity was used to characterize isolated RNA.

IP-to-MS. IP-to-MS analysis of plasma samples representing different patient and autoantibody subgroups was performed by Impact Proteomics, LLC (9). IP-to-MS replaces radiolabeled protein as a source of potential autoantigens with a reversible protein tag, called ProMTag. ProMTag has two key features: a reversible amine-reactive group and an irreversible beads capture functional group. Thus, whole-proteome K562 cell lysates were tagged with ProMTag where all proteins carry at least one ProMTag. This ProMTagged antigen pool was immunoprecipitated by patient antibodies bound to Protein A beads. After washing to remove non-target proteins, immunoprecipitated proteins were separated from immunoglobulins by binding to ProMTag-capture beads. The captured autoantigen target proteins were then subjected to in situ trypsin digestion; resulting peptides were then lyophilized and stored at -80°C. Twenty percent of yielded peptides were separated by an Evosep One HPLC system and subjected to tandem mass spectrometric analyses by a timsTOF Pro 2 mass spectrometer (Bruker).

CCP2 ELISA. To assess the presence of antibodies targeting citrullinated proteins, plasma samples (diluted 1:100 in diluent buffer) were separately assessed with a commercial IgG anti-CCP2 (cyclic citrullinated peptide 2) ELISA kit (Axis-Shield Diagnostics, United Kingdom) according to established protocol.

ANA testing. Selected plasma samples were assessed for anti-nuclear antibodies (ANA) using the Calbiotech (El Canon, CA) screening ELISA kit. Healthy control sera were used to establish an antibody index (OD450 of sample/calibrator cutoff) of 1.0 as the threshold for ANA positivity. All plasma samples were diluted 1:50 in diluent buffer.

Statistical considerations. Summary statistics included assessment of antibody frequency as well as concordance rates between conventional immunoprecipitation and IP-to-MS (that served as the gold standard). Clinical, demographic, and functional characteristics of serologically-defined subgroups were compared using Fisher's exact/Chi-square testing for dichotomous variables. Continuous variables were compared using student's t test for normally distributed data and Mann Whitney U test for non-parametric data.

Results

Protein and RNA Immunoprecipitation. Plasma samples derived from 483 INSPIRE trial subjects,15 patients with alternative interstitial lung diseases, and 12 healthy controls (without interstitial lung disease) were subjected to conventional protein immunoprecipitation (IP) using ^{35}S-methionine-labeled K562 cell lysate as potential target antigens according to the protocol outlined in the Methods section. Among the samples initially classified as IPF, 186 (38.5%) demonstrated clear protein bands of interest (Fig. 1 **and Supplemental Fig. 1)**, while 119 other samples (28.6%) yielded equivocal bands. Four additional samples (out of 483) demonstrated anti-CCP2 antibodies by ELISA (variable titer), and the remaining 174 samples did not have evidence of autoantibodies by protein IP or CCP2 ELISA. RNA IP of 100 samples selected on the basis of protein IP patterns (potentially indicative of RNA-binding autoantigens) provided additional detail regarding autoantibody specificity (Fig. 2 **and Supplemental Table 1**). Based on these collective protein and RNA IP data, as well as the CCP2 ELISA results, we classified the INSPIRE trial samples into the following autoantibody-based subgroups (**Table 1**): **Subgroup 1-A**: antibodies targeting amino-acyl tRNA synthetases, topoisomerase I, TH/TO, Ro60, U3 RNP, or other snRNPs–n = 134; **Subgroup 1-B**: antibodies targeting an unknown 56 kDa antigen; n = 27; **Subgroup 1-C**: antibodies recognizing an undefined 31/35 kDa doublet–n = 25; **Subgroup 2**: anti-CCP2–n = 4; **Subgroup 3**: equivocal/other protein bands–n = 119; and **Subgroup 4**: negative autoantibody screen–n = 174. This classification scheme encompasses patients with autoantibodies of interest (n = 186, **Subgroups 1A-1C**), those with bands of unknown identity (n = 119, **Subgroup 3**), and those without evidence of autoantibodies (n = 174, **Subgroup 4**).

Because the conventional, gel-based protein IP showed a diversity of novel protein bands that did not always correlate with known autoantigens–particularly in subgroups 1-B, 1-C, and 3–IP-to-MS was used to both confirm conventional IP autoantibody determinations and to identify novel autoantigens. Based on availability of remaining plasma, IP-to-MS analysis was performed on 75 of the 483 INSPIRE trial samples previously assessed by gel-based IP: 68 samples yielding putative autoantigens by conventional IP and 7 samples that did not yield identifiable bands by conventional IP [Note that five samples were run as duplicates, bringing the total number of patient samples assessed by IP-to-MS experiments to 80]. In addition, 16 sera derived from healthy subjects without interstitial lung disease were assessed by IP-to-MS as negative controls. A heatmap of the results was generated to provide a full display of the IP-to-MS results, plotting patient sample ID horizontally versus MS- identified proteins vertically (Fig. 3A **and Supplemental Table 2**). The top eleven protein rows appear to span the majority of

samples and likely represent a set of common non-specific binding proteins. Conversely, the remaining protein rows represent putative autoantigens that were immunoprecipitated by individual subjects, sometimes in sample-specific vertical clusters not found in healthy control samples.

As shown in **Fig. 3B** and **Table 2**, IP-to-MS revealed numerous autoantibodies (in multiple patient samples) that target known autoantigens including tRNA synthetases (asparaginyl-, threonyl-, and tryptophanyl-tRNA synthetase), topoisomerase I, TH/TO, centromere V, and Ro60. These autoantigens are indicators of autoimmune diseases such as myositis, scleroderma, Sjogren's, and systemic lupus erythematosus. In addition, other known autoantigens were detected in single patient representatives, including X-ray repair cross-complementing proteins, exosome proteins, and RNase P subunits (Fig. 3A). Gel-based IP revealed previously unidentified 56 and 31/35 kDa proteins. IP-to-MS identified the commonly immunoprecipitated 56 kDa protein as Annexin A11 and suggested that the frequently observed 31/35 kDa bands could be prohibitin 1/prohibitin 2 (Fig. 3C). Other potential autoantigens of interest detected in several samples, but not found in control samples, included elongation factor 1-alpha 2 (n = 3), CENPV (n = 8), DLAT, a component of the mitochondrial pyruvate dehydrogenase complex (n = 9), heterogenous ribonucleoproteins C1/C2 (n = 10), and heat shock protein 71 (n = 15) (Fig. 3C). As a complementary assessment of additional anti-nuclear antibodies, we performed ANA testing (using a commercial ELISA) on the subset of samples listed in **Table 2**. Of note, the results of ANA testing were highly discordant from the IP-to-MS analysis, as only 7 samples demonstrated low levels of anti-nuclear antibodies.

Because conventional, gel-based IP is widely considered to be the gold-standard for autoantigen classification, we compared the results from conventional IP versus IP-to-MS for the 80 INSPIRE samples analyzed by both methods (**Tables 2 and 3**). The results were compared on two levels: a) gel band apparent molecular mass versus MS-identified protein mass (**Table 2**) and b) expert interpretation of conventional IP versus MS protein identification (**Table 3**). A concordance metric was established where agreement between methods was deemed "good" if one or more MS-identified protein had a band of similar apparent molecular mass on the corresponding IP protein gel. The degree of agreement was characterized as "fair" if there was no direct mass agreement, but the observed protein gel band could be explained by known breakdown protein products of the MS identified protein. Three proteins stood out in this category: APOB, VWF, and IHIT4 (each of which are known to experience complex breakdown patterns). Samples with no demonstrable concordance between gel-based IP and IP-to-MS were ranked as "poor". Finally, there were a few instances where IP-to-MS identified more proteins than indicated by conventional IP.

Overall, there was very good agreement between the gel-based protein bands and IP-to-MS assignments, where 75% (60/80) of the sample displayed good agreement (**Table 2**). Fifteen percent (12/80) of the samples were categorized as fair, as larger scale analysis will be required to determine if the observed gel bands are indeed breakdown products of the MS identified protein. Only 5% (4/80) of the comparisons were found to have poor agreement. Likewise, 5% (4/80) samples yielded more MS protein identifications than suggested by conventional IP banding patterns.

Conventional, gel-based IP interpretation relies on visual inspection of protein gel banding patterns relative to known standards. In this study, autoantigen identification was classified as “probable” or “possible” by an expert with more than 30 years of experience (NF). To assess concordance between these expert assessments of conventional IP versus the newer IP-to-MS profiling, we compared proteins identified by both methods (**Table 3**). Of the 33 probable calls, 23 (70%) agreed with MS data. In instances where the experts made possible assignments, none of the 41 possible calls agreed with the MS identifications. However, even in cases where mass spectrometry analysis did not confirm the interpretation of conventional IP, alternative autoantigens were routinely identified.

Clinical characteristics of Autoimmune ILD and IPF subpopulations. To determine the clinical implications of this immunoprecipitation analysis, we divided the INSPIRE patient cohort by autoantibody profile (*as determined by conventional IP*) into the following *clinical* subsets: 1) Autoimmune ILD (AILD; antibody subgroup 1-A; n = 134) and 2) IPF patients without immunoprecipitation-based evidence of autoantibodies (antibody subgroup 4; n = 174). Samples with indeterminate profiles (antibody subgroup 3) and those associated with antigens of unknown significance (antibody subgroups 1-B and 1-C) were omitted from this analysis. Review of **Table 4** demonstrates similar clinical/demographic characteristics in individuals classified as AILD versus IPF by autoantibody profile (antibody subgroup 1A (AILD) versus subgroup 4 (IPF)). Specifically, there were no significant differences in sex distribution, race, or smoking history between AILD and IPF subjects. More importantly, there were no significant differences in baseline parameters of FVC percent predicted, DLCO percent predicted, or 6MWD between autoantibody subsets of AILD versus IPF. Correspondingly, the percentage of patients requiring supplemental oxygen was similar between AILD and IPF subgroups. Correlations between these serologically-defined subgroups and various outcome parameters (e.g., percent reduction in FVC over time, time to respiratory-related hospitalization, survival) did not show any significant differences.

Discussion

Among nearly 500 subjects enrolled in the INSPIRE trial based on a disease classification of IPF, 190 (39%) had evidence of probable/definite autoantibodies indicative of an underlying autoimmune process [186 with autoantibodies defined by IP (categories 1A-1C), 4 with positive CCP2 ELISA]. Mass-spectrometric analysis of selected immunoprecipitates substantiated these observations and, in some cases, identified novel autoantigens—demonstrating the utility of the IP-to-MS work flow. These collective findings exceeded our initial estimates of seroprevalence in this population and fully support our underlying hypothesis that a substantial portion of patients classified as IPF have an underlying autoimmune diathesis marked by autoantibodies targeting antigens previously associated with connective tissue diseases. In fact, while a number of the samples tested by conventional IP immunoprecipitated putative autoantigens of unknown identity (52/483, 11%; subgroups 1-B and 1-C), a sizeable percentage targeted defined antigens associated with the anti-synthetase syndrome, systemic sclerosis, or autoimmune overlap syndromes (134/483, 28%; subgroup 1-A).

While previous studies have, in fact, suggested the presence of occult anti-synthetase and other CTD-associated autoantibodies in patients with idiopathic interstitial pneumonia (IIP) (10–12), the rate of seroprevalence in the INSPIRE cohort of putative IPF patients was substantially higher than in these earlier analyses. Moreover, among more recent studies documenting the presence of autoantibodies in rigorously classified cohorts of IPF (13–17), none have consistently identified these CTD-associated autoantibodies—further demonstrating the specificity of autoantibodies detected in our study for classifiable, systemic autoimmune diseases.

What is equally striking about our results is that patients enrolled in INSPIRE had to have radiographic and/or biopsy evidence of UIP rather than NSIP, COP, or other histopathologic subtypes more likely to be associated with CTD-ILD or alternative inflammatory etiologies. Given the potential implications for clinical trials of anti-fibrotic and other emerging therapeutic agents, appropriate clinical and serological screening for underlying autoimmune disease is of paramount importance. In the INSPIRE trial, patients did not undergo routine laboratory testing for ANA, ANCA, CCP, or other commercially available autoantibodies. Although such screening would undoubtedly have identified some of the patients with autoimmune characteristics, it is important to point out that ANA testing alone would not detect a substantial portion of patients with myositis-associated antibodies targeting cytoplasmic antigens such as aminoacyl-tRNA synthetases—particularly when using ELISA-based approaches rather than indirect immunofluorescence (IIF). In fact, ANA testing of the 75 plasma specimens subjected to IP-to-MS analysis (**Supplemental Table 2**) yielded only 7 low-moderate titer ANA-positive samples (including only 1/7 samples identified as synthetase-positive by IP-to-MS in this subset). Unfortunately, the limited availability of patient plasma precluded more comprehensive comparative assessment of ANAs in our study.

A key question is how more detailed serological classification of patients with IIP/IPF might impact assessment of clinical outcomes and response to immunomodulatory therapies such as IFN-gamma-1β. In fact, incorporation of serological profiles as a distinct domain in the previously developed clinical construct of Interstitial Pneumonia with Autoimmune Features (IPAF) (18) has yielded mixed results, with some studies demonstrating an intermediate phenotype between IPF and CTD-ILD and others showing no difference in clinical outcomes relative to IPF (19–24). Within cohorts classified as IPF, a growing number of studies have revealed antibodies recognizing a range of cytoplasmic, cytoskeletal, and/or structural proteins (13–17). Although it remains unclear whether these antibodies mark forme fruste manifestations of autoimmune disease, these studies do suggest that certain antibody specificities such as anti-THBS1 predict worse clinical outcome/reduced survival (17).

In our analysis of the INSPIRE trial, we were unable to detect statistically significant differences in clinical outcome parameters (such as reduction in percent predicted FVC, time to respiratory-related hospitalization, or survival) between patients reclassified as AILD (subgroup 1-A) and the autoantibody negative IPF subgroup following treatment with IFN-gamma-1β. When interpreting these preliminary data, however, it is important to consider that our study was not designed to assess differences in clinical outcomes, in part because of limitations in sample availability that precluded serologic

assessment of the entire study cohort. Furthermore, designation/classification as AILD based on IP-determined autoantibody profile alone was likely flawed, as shown by the IP-to-MS data in **Table 2** indicating that a modest percentage of autoantibodies were incorrectly identified by conventional IP and other potential autoantibodies were not detected by standard approaches. Equally important, combining different autoantibody subsets marking specific phenotypes may have obscured altered biological responses to IFN-gamma-1β in the INSPIRE trial. As evidenced by the data presented in **Table 2**, IP-to-MS will serve as an invaluable tool that can overcome many of the technical limitations with conventional IP (use of radioactivity, reliance on banding patterns rather than mass spectrometry-based profiling) to help address these issues and permit more precise classification of target autoantigens as well as associated disease phenotypes—a key consideration given the emerging paradigm that serologically-defined endotypes in IIP/IPF may predict biological behavior and clinical course (17). It is important to point out, however, that even this more precise disease classification scheme may not have altered our analysis of outcomes in the INSPIRE trial, as there is no *a priori* reason to expect that IFN-gamma-1β would be more effective in correctly designated AILD, particularly given the complex role of TH1/IFNγ-driven immune responses [which can indirectly promote fibrosis via M1 macrophage-derived cytokines such as TNFα and IL-1β (25)] in different stages and subtypes of CTD-ILD.

Beyond these issues, another constraint on our findings is that the INSPIRE radiology database failed to use the same participant numbers for their initial and follow-up scans. More importantly, different databases were developed for serological markers that were not linked to the participant numbers in the imaging biobank. As a result, we could only compare our results to the overall imaging analysis conducted by the study's blinded radiologists, which noted that "definite fibrosis" was present in only 61% of the scans (301/491). We acknowledge that the inability to correlate identified biomarkers with their paired images hinders the application of our findings. The trial's impact from this omission should raise awareness of the critical importance of linked database entries in clinical studies. However, none of these limitations diminish the significance of our key observation that a substantial percentage of patients with clinical and radiologic features of IPF possess autoantibodies suggestive of underlying autoimmunity. With the advent of our novel IP-to-MS approach that has been adapted to a high throughput, 96-well format (9), precise molecular characterization of autoantigen targets will become even more feasible—which will be crucial in defining subphenotypes of CTD-ILD as well as IPF.

Given the range of clinical data available from other completed clinical trials in IPF, retrospective identification/classification of autoimmune ILD should permit preliminary estimates of therapeutic efficacy for pirfenidone and other anti-fibrotic agents in this alternative disease subset [that will become better defined as we improve our understanding of clinical features associated with different autoantibodies (such as anti-annexin A11) identified in this cohort]. At the same time, more detailed serological profiling and exclusion of non-IPF patients from earlier data analyses may lead to revised assessments of therapeutic efficacy in IPF (or serologically-defined subsets of IPF)—underscoring the importance of correct disease classification. To address the clinical response of subjects with non-IPF progressive pulmonary fibrosis (PPF), recent clinical trials encourage attention to the heterogeneous populations that develop fibrotic lung disease (26, 27). By extension, the findings of the present

retrospective study have clear implications for future clinical trials in which proper clinical and serological classification will be required to ensure appropriate patient selection/enrollment and allow more accurate assessment of therapeutic efficacy.

Declarations

FUNDING AND DISCLOSURES

Funding and statistical support were provided by Roche Genentech, Inc. Samples and trial data were collected by InterMune, Inc. prior to its acquisition by Roche Genentech, Inc.

Ethics approval, consent to participate, and consent for publication. The INSPIRE trial adhered to principles outlined in the Declaration of Helsinki, and all enrolled patients provided consent to participate as well as consent for publication of de-identified data encompassing clinical/demographic characteristics and associated biospecimens. Protocols and consent forms were approved by Institutional Review Boards or Ethics Committees representing individual institutions/clinical centers enrolling patients into the INSPIRE trial.

Competing interests. The authors have no competing interests to declare beyond research funding from Roche Genentech, Inc. provided to MKG and DPA. Both CB and BT are employees and shareholders at Roche Genentech, Inc., but have no ties to the INSPIRE clinical trial or the use of IFN-gamma-1b as a therapeutic agent.

Author Contribution

Data acquisition: MKG, CB, SP-S, JLS, LH, SB, SE, JSM, DPAData analysis/interpretation: BT, NF, JSM, DPADrafting manuscript: MKG, JSM, DPASources of support: Roche Genentech, Inc. (MKG, DPA)

Data Availability

All research data generated during the preparation of this manuscript is provided in supplementary figures/tables.

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Figures

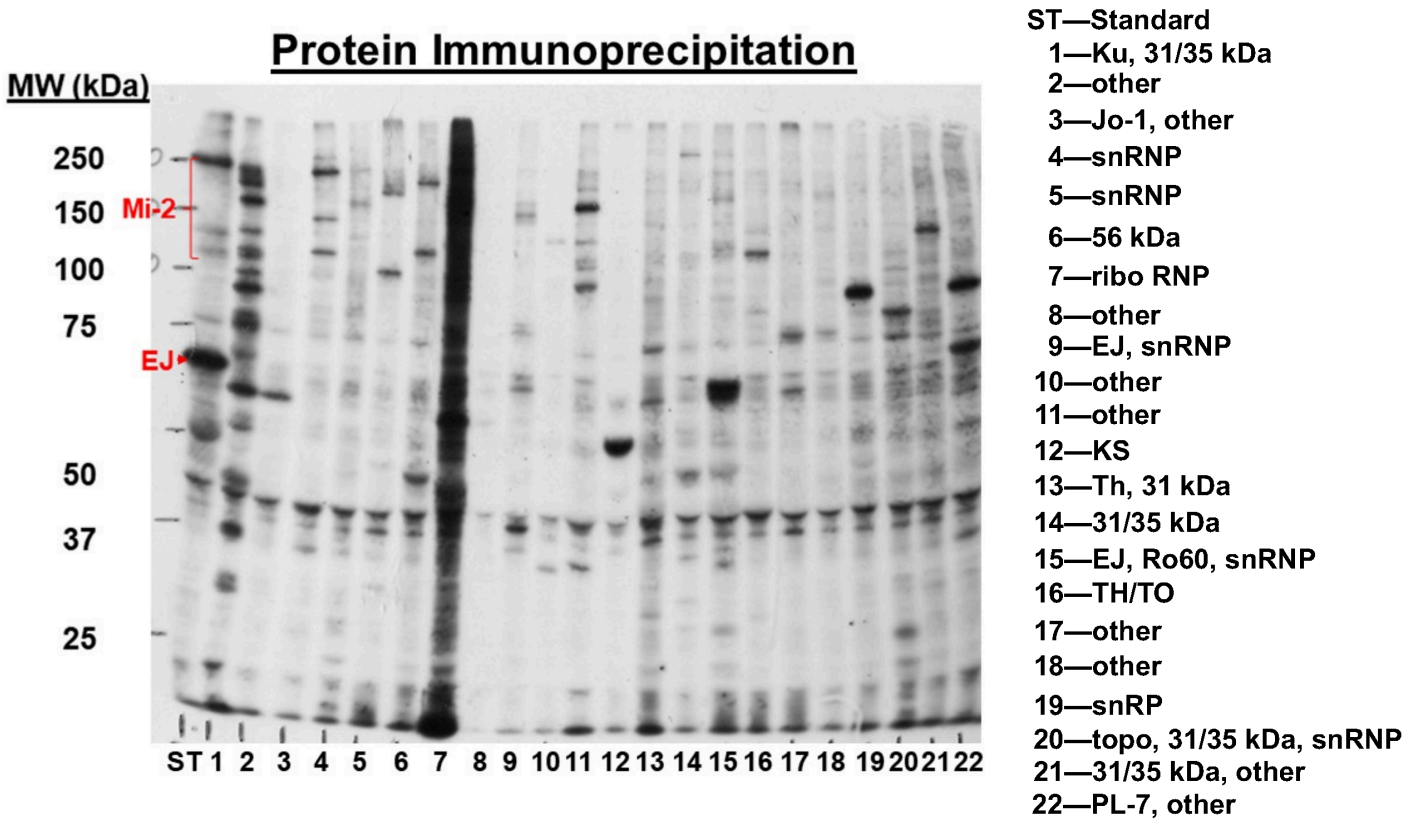

Figure 1

Protein Immunoprecipitation. The depicted autoradiogram demonstrates K562-derived radiolabeled proteins immunoprecipitated by different sera; protein identities are listed on the right.

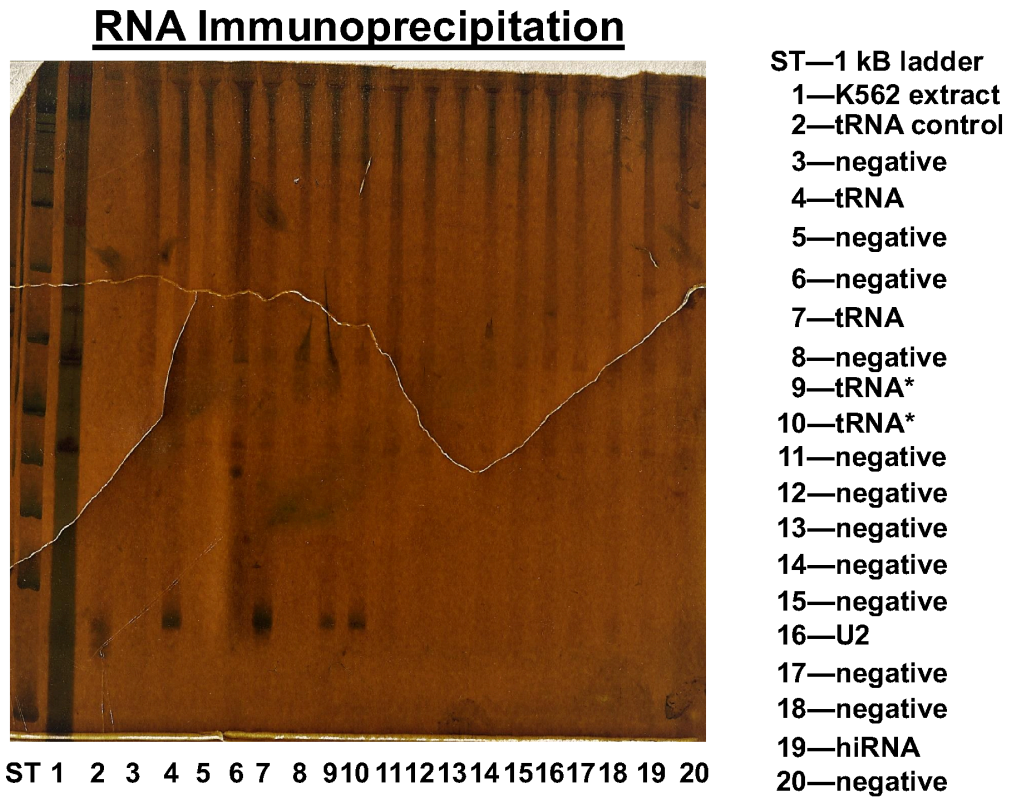

Figure 2

RNA Immunoprecipitation. The silver-stained gel shows different types of RNA complexes (listed at right) immunoprecipitated by individual sera; *duplicate samples.

Figure 3

IP-to-MS of 80 selected INSPIRE samples. (A) Shows the full heatmap of proteins identified by IP-to-MS in specimens derived from INSPIRE participants (n=80) and healthy controls (n=16). The intensity scale represents the $\log_2$ of spectral count data for all proteins identified. A very stringent cutoff was applied to only include proteins with a spectral count of at least two, where each spectral count represents a sequenced peptide associated with a unique protein and can be used as a proxy for relative protein

abundance. The protein rows were assorted according to a pattern clustering algorithm. (B) Shows ILD antigens associated with myositis, SSc, and Lupus. (C) Shows antigens not typically associated with ILD.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- OnlineSupplementAschermanfinal.pdf
- SupplementalFigure1Ascherman.pdf
- SupplementalTable1.xlsx
- SupplementalTable2.xlsx