case reports

Recurrent Myocarditis Induced by Immune-Checkpoint Inhibitor Treatment Is Accompanied by Persistent Inflammatory Markers Despite Immunosuppressive Treatment

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INTRODUCTION

Immune checkpoint inhibitors (ICIs) have significantly improved advanced cancer outcomes.[1,2] Although ICIs unleash antitumor immune responses, negating natural immune inhibitory mechanisms can result in the development of self-reactive immune cells and an array of immune-related adverse events (irAEs).[3] Although reports of serious myocarditis have emerged,[4-9] data on long-term outcomes following an episode of ICI-associated myocarditis are limited and molecular analyses on surviving patients are lacking. Here, we report a case of recurrent myocarditis in a patient previously treated with a programmed death ligand 1 (PD-L1) inhibitor (durvalumab), an ICI that blocks the binding of PD-L1 to programmed cell death protein 1 and CD80.[10] Molecular investigations revealed cytokine modulations suggestive of a sustained T-helper 1 (T_h1)-like cellular immune response as well as unique antigen-bound serum autoantibodies, highlighting a potential role for T_h1-cell-dependent induction of autoantibody production in irAEs.

CASE

Initial Presentation

A 67-year-old woman presented to the emergency department with severe sudden-onset chest pain 2 days after receiving the 12th cycle of PD-L1 inhibitor (48 weeks after starting ICI) for the treatment of metastatic urothelial carcinoma (Fig 1A). She had no previous history of cardiovascular disease but was an ex-smoker with a 20 pack-year history. She had no other known cardiovascular risk factors and no previous history of autoimmune disease. She was previously treated for remote triple-negative right-sided breast invasive ductal carcinoma with surgery; cyclophosphamide, epirubicin, and 5-fluorouracil chemotherapy; and radiation. Subsequently, she was diagnosed with multifocal transitional cell carcinoma of the kidney 2 years before this emergency department admission and was treated with nephroureterectomy and pelvic node dissection. PD-L1 inhibitor was commenced after subsequent investigations revealed intra-abdominal metastasis. Her treatment was largely uneventful, with only a noted diagnosis of hypothyroidism after receiving the 7th cycle of PD-L1 inhibitor, which was treated with hormone replacement therapy without interruption of ICI.

Although the patient was hemodynamically stable, cardiac laboratory investigations revealed high-sensitivity troponin I levels were elevated at 4,114 ng per L (reference range, < 26 ng per L), and brain natriuretic peptide levels (BNP) were elevated at 2,275 pg per mL (reference range, < 100 pg per mL) (Figs 1B and 1C, Data Supplement). Electrocardiogram (ECG) displayed diffuse ST elevation. She was transferred to the catheterization lab, and coronary angiogram revealed patent coronary arteries. 2D echocardiogram demonstrated severely decreased biventricular systolic function, with a left ventricular ejection fraction (LVEF) of 20%, right ventricular (RV) fraction area change of 22%, and a left ventricular (LV) thrombus (Fig 1D and Video 1). With a history of ICI treatment, together with clinical, ECG (Fig 2A), and imaging findings, a diagnosis of ICI-associated myocarditis was made. Treatment with intravenous methylprednisolone (2 mg/kg/d), furosemide, beta-blocker, angiotensin-converting enzyme inhibitor, and low-molecular-weight heparin was initiated. Cardiovascular magnetic resonance (CMR) imaging confirmed severe biventricular dysfunction, mild pericardial effusion, and demonstrated late gadolinium enhancement (LGE) involving the basal and apical segments predominantly in a subepicardial distribution (Video 2, Data Supplement). Follow-up ECG remained abnormal with low-voltage QRS complex as well as persistent ST elevation (Fig 2B).

The patient was discharged home in stable condition on 90 mg oral prednisone with a plan to taper and discontinue in 8 weeks. ICI therapy was discontinued, and low-molecular-weight heparin was prescribed for 3 months to treat the LV thrombus. A chest computed tomography scan before discharge displayed no

ASSOCIATED CONTENT

Data Supplement
Video

Author affiliations and support information (if applicable) appear at the end of this article.

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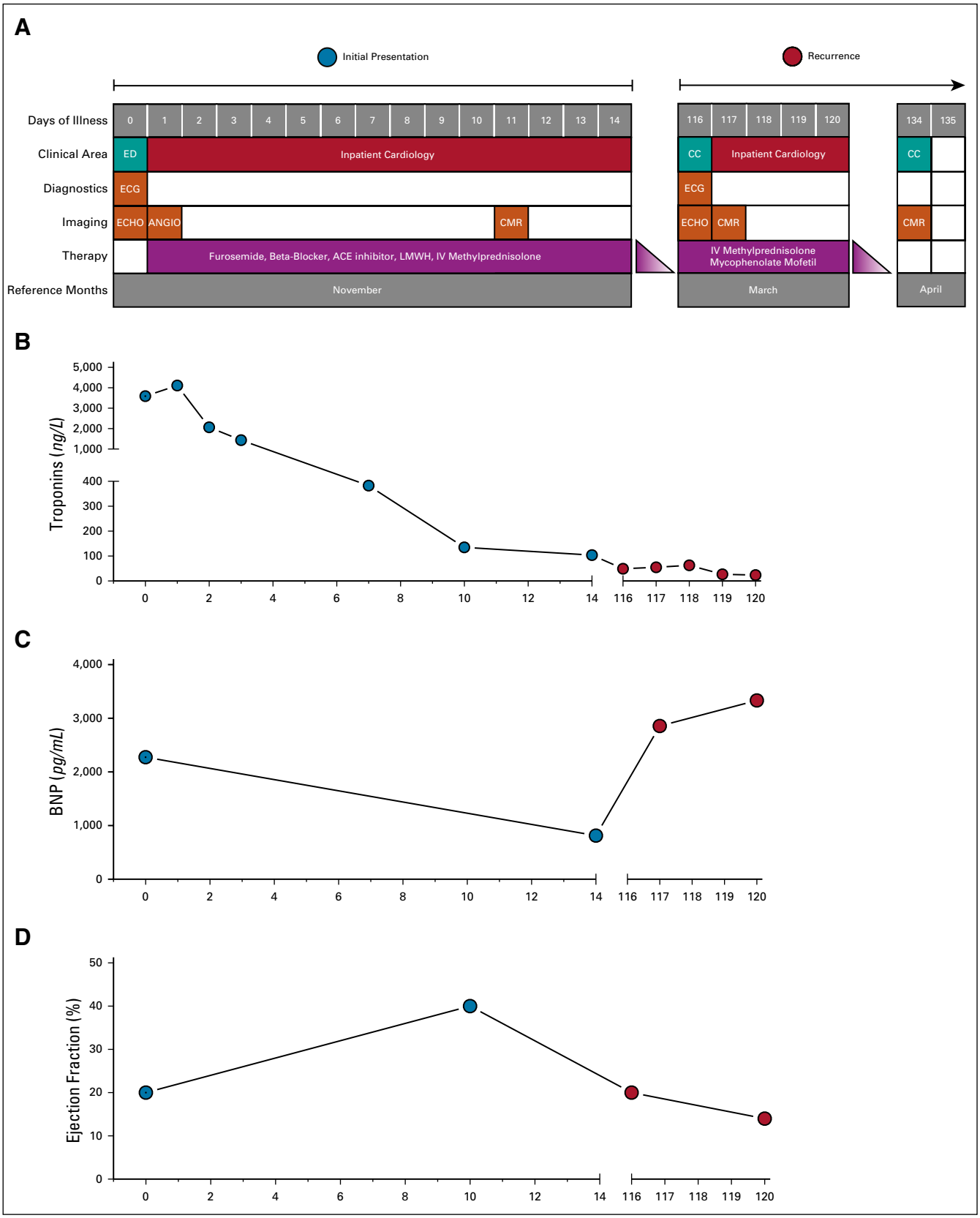

FIG 1. Cardiac laboratory data during initial immune checkpoint inhibitor–associated myocarditis and at recurrence reveal elevated brain natriuretic peptide (BNP) but not troponin I. The course of clinical presentation, diagnostic workflow, and key timepoints of immunosuppressive initiation (A). Each box represents a day since the initial clinical presentation with the break in the timeline representing the recurrent presentation. The initial presentation

Echocardiogram (apical four chamber view) of the patient demonstrating biventricular dysfunction as well as a focused apex view with contrast showing a thrombus (admission).

evidence of pulmonary emboli, whereas whole-body computed tomography scanning showed no evidence of metastatic disease. There was significant clinical recovery 1 month after discharge, with echocardiography revealing partial recovery of LV function (LVEF = 40%; global longitudinal strain = −17.1%); however, RV systolic function remained severely reduced (RV fraction area change 17%, Data Supplement). ECG remained abnormal with low-voltage QRS, however, now with right bundle branch block (Fig 2C).

Recurrent Presentation

Four months after the initial event, and without resumption of ICI treatment, the patient presented to the cardiology clinic with extreme fatigue. A 2D echocardiogram showed a drop in LV systolic function (2D LVEF = 20%; Data Supplement). Repeat ECG showed persistence of low-voltage QRS and right bundle branch block (Fig 2D). Troponin was modestly elevated at 49 ng/L, and BNP was elevated at 2,857 pg/mL. She was admitted and a subsequent CMR confirmed the severity of LV dysfunction, multiple new LV thrombi, larger pericardial effusion, and a mild interval worsening of nonischemic pattern LGE (Data Supplement). A cardiac biopsy (Figs 2E and 2F) confirmed the diagnosis of myocarditis with predominantly T cells (Fig 2G) and macrophages (Fig 2H); polymerase chain reaction was negative for cardiotropic viruses.[11] Given the biopsy result, she was diagnosed with recurrence of ICI-associated myocarditis. More aggressive treatment was undertaken, this time with 1,000 mg pulse methylprednisolone in combination with mycophenolate mofetil (MMF) for immunosuppression. She was discharged home on oral prednisone (instructions to taper) and MMF. However, two weeks following oral prednisone discontinuation, she was readmitted with decompensated heart failure where CMR displayed persistence of diffuse LGE and severe biventricular dysfunction. She deteriorated and subsequently succumbed to heart failure a year-and-a-half later.

METHODS

The patient was enrolled in this research ethics board-approved case report at the University Health Network after obtaining informed written consent. Biomarker examinations were performed once before recurrent ICI myocarditis (January 2018) and subsequently throughout the patient's recurrence (March onward; Fig 1A). Next-generation sequencing (ImmunoSeq), cytokine multiplex assays, targeted microRNA profiling, and a mass spectrometry-based assay to identify serum autoantibody complexes with their cognate antigens were performed (for a detailed description of the methods, see the Data Supplement).

RESULTS

Analysis of the distribution, clonality, and diversity of T-cell receptors pre-recurrence (January) and during treatment for recurrence of myocarditis (March onward) with immunosuppressants revealed limited clonal modulation (Data Supplement). Multiplex analysis of cytokines (Luminex, Austin, TX) in serum revealed remarkably high concentrations of T-cell–derived cytokines during recurrence despite withdrawal of ICI therapy and initiation of immunosuppressants. Substratifying by T-helper (T_h) cell subtype revealed that there was an apparent dominance of T_h1-cell–enriched cytokines over T_h2-cell–enriched cytokines (Figs 3A and 3B; Data Supplement).

Quantification of 92 microRNAs (Fluidigm, San Francisco, CA) with known inflammatory and cardiac roles revealed two microRNAs (miR-130a-3p and miR-122-5) that displayed consistent upregulation and downregulation, respectively, in parallel with circulating cytokine abundance. Increases in miR-130a-3p (Figs 3B and 3C) appeared to correlate with declines in interleukin (IL)-4,[12] whereas decreases in miR-122-5p, a noted negative regulator of IL-1, appeared to be associated with elevations in IL-1 (Figs 3A and 3C).[13] Principal component and pathway analysis of differentially regulated microRNAs (cross-timepoint comparison) revealed modulation during treatment course (Data Supplement).

To assess the downstream effects of a potentially aberrant T_h1 cell response, we examined antigen-bound serum autoantibodies. It was noteworthy that the patient experienced hypothyroidism after receiving ICI as we identified antithyroglobulin autoantibody across all sample collection points (Roche electrochemiluminescent assay) (Data Supplement). A mass spectrometry-based assay[14-16] to identify serum autoantibodies complexed with their cognate antigens detected thirteen autoantibodies related to cardiac and coagulative pathway (Fig 3D; Data Supplement). These were elevated before recurrence of myocarditis and remained present during the course of treatment. Among these, nine candidate autoantibodies were previously linked to autoimmune disease.[17-19]

DISCUSSION

This is the first report and detailed molecular examination of recurrent ICI-associated myocarditis without ICI re-exposure.

FIG 1. (Continued). was marked by strikingly elevated troponins (B), elevated BNP (C), and by low ejection fraction (D). By contrast, the recurrent presentation, although displaying low ejection fraction and elevated BNP (C and D), did not display markedly elevated troponin levels (B). ACE, angiotensin-converting enzyme; ANGIO, angiogram; CC, cardiology clinic; CMR, cardiovascular magnetic resonance; ECG, electrocardiogram; ECHO, echocardiogram; ED, emergency department; IV, intravenous; LMWH, low-molecular-weight heparin.

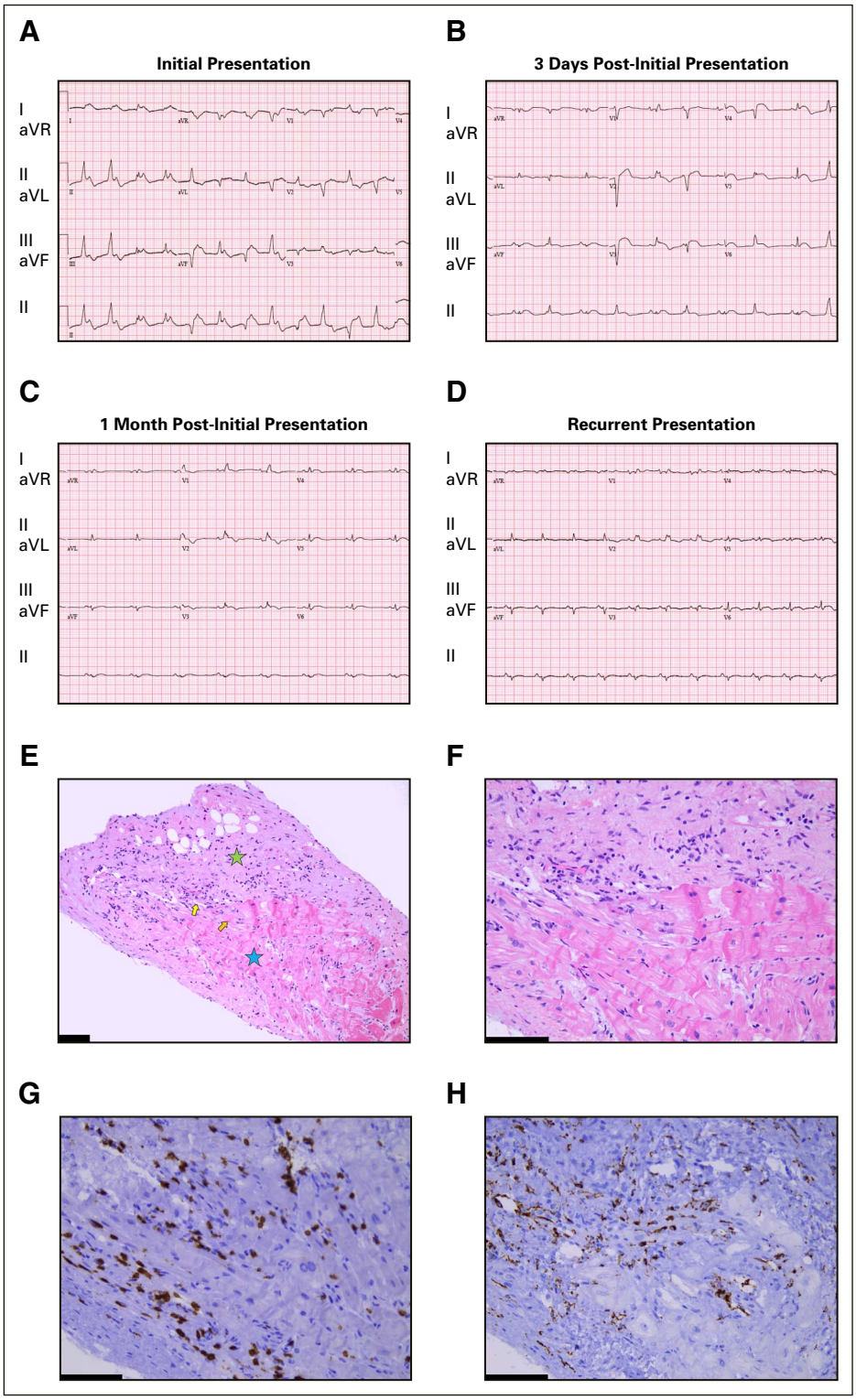

FIG 2. Results of ECG (initial and recurrence) as well as immune effects in cardiac muscle at recurrence. ECG at the time of initial presentation shows sinus tachycardia and diffuse ST elevation (A). Follow-up ECG after initial presentation shows persistence of ST elevation and development of low-voltage ECG (B). ECG one month after initial presentation shows low-voltage ECG and development of right bundle branch block (RBBB, C). ECG at recurrence displayed persistence of low-voltage QRS and RBBB without new acute changes (D). Myocardial biopsy (E) demonstrated mixed inflammation (yellow arrow) with some associated myocyte damage (orange arrow) and some healing injury (green star) near relatively uninvolved myocardium (cyan star); higher-power image is also shown (F); hematoxylin and eosin staining; digital acquisition; scale bars as shown = 100 μm. Immunohistochemistry demonstrated that most of this infiltrate was composed of CD3-positive T cells (G, stained cells in brown) and CD68-positive macrophages (H, stained cells in brown). aVR, augmented vector right; aVL,augmented vector left; aVF, augmented vector foot.

CMR showing the presence of severe biventricular dysfunction, mild pericardial effusion, and a short axis late gadolinium enhancement (LGE) stack showing LGE in a subepicardial distribution involving the basal and apical segments (admission).

Although irAEs often develop within the first few weeks to months after initiation of treatment, these events can present at any time, including after cessation of ICI therapy. This case highlights the importance of regular clinical cardiovascular

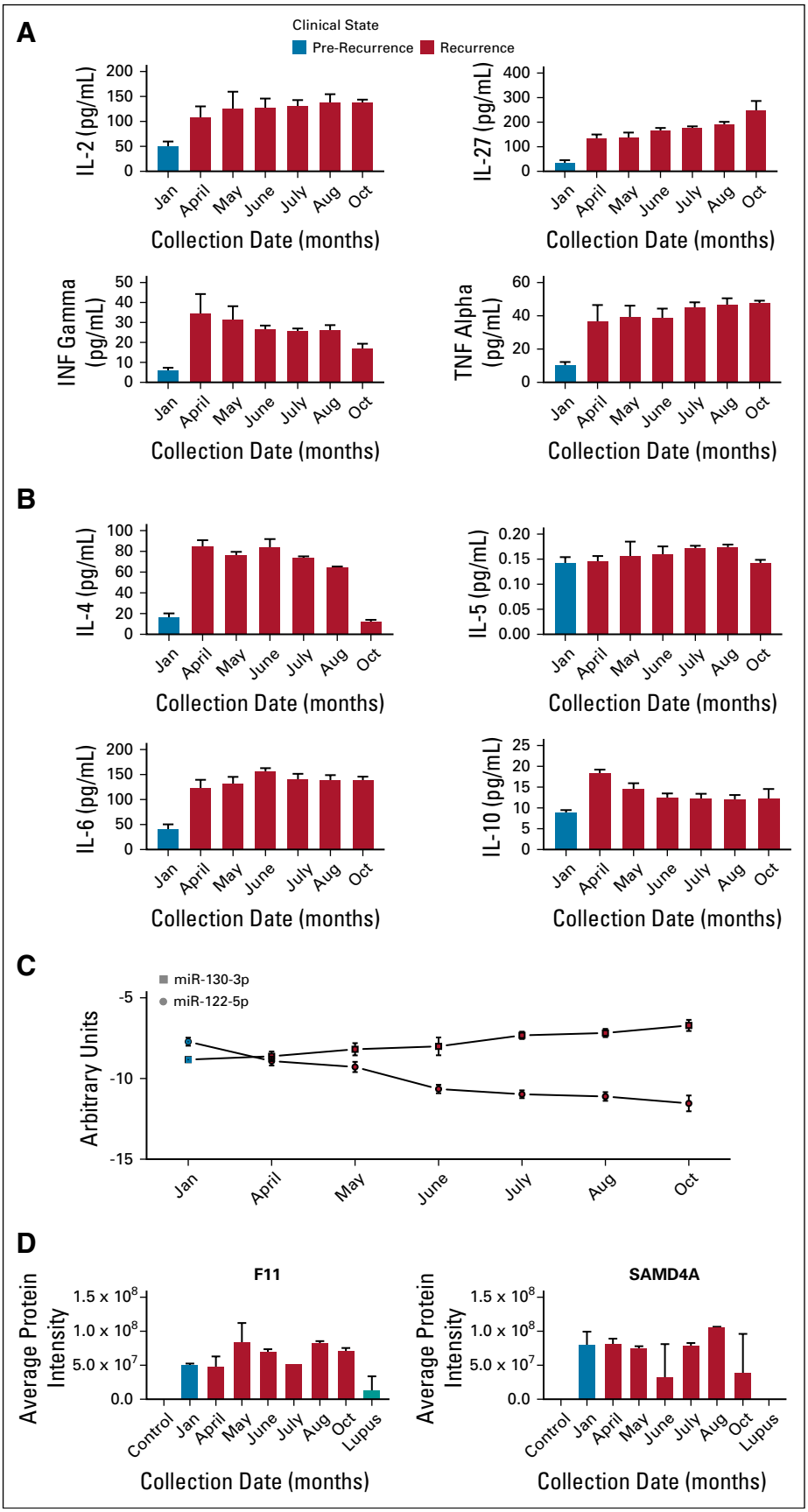

FIG 3. Circulating cytokine profiles, miRs, and the presence of antigen-bound serum autoantibodies reflect a T_h1-dominated response sustained throughout the recurrent presentation. Luminex-based analysis of circulating inflammatory cytokines revealed consistently elevated responses enriched in T_h1 cell cytokines (A) in contrast to T_h2 cell cytokines (B). Two miRs related to cardiac function and inflammation displayed consistent trends in expression with miR-130-3p trending upward and miR-122-5p trending downward (C). Serum autoantibody complexes were observed consistently in the programmed cell death protein-1 ligand 1–induced fulminant myocarditis patient compared to age- and sex-matched control patients (n = 7), and a patient with myocarditis from systemic lupus erythematosus (n = 1; D). Serum autoantibody complex data are displayed as relative expression (average protein intensity) with data presented as the mean ± the standard deviation. MiR and cytokine data are presented as the mean ± the standard deviation of technical triplicates. Some error bars may not be visible due to the scale. F11, coagulation factor XI; IL, interleukin; INF, interferon; miR, microRNA; SAMD4A, protein Smaug homolog 1; TNF, tumor necrosis factor.

surveillance after an episode of myocarditis, and the necessity for rapid and effective immunosuppression.[4] Treatment with high-dose glucocorticoids and MMF appeared to improve symptomology; however, it may have failed to affect the underlying etiology as BNP levels remained marginally abnormal while high levels of inflammatory cytokines and autoantibodies persisted. It is possible that in addition to generating autoreactive T cells against cardiac epitopes (a proposed cause of ICI-mediated myocarditis), ICIs may also induce T_h1-dependent pathogenic autoantibody production (Data Supplement).

Patients presenting with symptoms suggestive of ICI-related myocarditis present a significant diagnostic challenge. Cardiac troponins can be negative in cases of myocarditis.[20] Indeed, in this case, troponins were only intermittently and mildly elevated during myocarditis recurrence. The diagnostic value of echocardiography and ECG is often limited, with findings suggestive of myocarditis either absent initially or lacking specificity.[4,6] The addition of CMR may be beneficial owing to its potential to detect inflammation, edema, and fibrosis within myocardial tissue. However, LGE alone has low sensitivity and accuracy in diagnosing chronic or recurrent myocarditis, and CMR is difficult in hemodynamically unstable patients.[21]

Although the recovery of LV function and resolution of symptoms followed by a separate recurrence of symptoms consistent with acute myocarditis is suggestive of a distinct myocarditis event, one other possibility could include the persistence of subclinical inflammation between episodes, leading to smouldering myocarditis and subsequent heart failure. This case nonetheless raises awareness that serious irAEs can occur after ICI discontinuation, and without re-exposure, reinforcing the importance of cardiovascular surveillance after an episode of ICI-associated myocarditis along with detailed diagnostic work-ups. As such, longer duration of immunosuppression after an episode of ICI-associated myocarditis may be warranted.

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