Neutralizing anti–IL-1 receptor antagonist autoantibodies induce inflammatory and fibrotic mediators in IgG4-related disease

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Background: IgG4-related disease (IgG4-RD) is a fibroinflammatory condition involving loss of B-cell tolerance and production of autoantibodies. However, the relevant targets and role of these aberrant humoral immune responses are not defined.

Objective: Our aim was to identify novel autoantibodies and autoantigen targets that promote pathogenic responses in IgG4-RD.

Methods: We sequenced plasmablast antibody repertoires in patients with IgG4-RD. Representative mAbs were expressed and their specificities characterized by using cytokine microarrays. The role of anti–IL-1 receptor antagonist (IL-1RA) autoantibodies was investigated by using in vitro assays.

Results: We identified strong reactivity against human IL-1RA by using a clonally expanded plasmablast-derived mAb from a patient with IgG4-RD. Plasma from patients with IgG4-RD exhibited elevated levels of reactivity against IL-1RA compared with plasma from the controls and neutralized IL-1RA activity, resulting in inflammatory and fibrotic mediator production in vitro. IL-1RA was detected in lesional tissues from patients with IgG4-RD. Patients with anti–IL-1RA autoantibodies of the IgG subclass had greater numbers of organs affected than those without anti–IL-1RA autoantibodies. Peptide analyses identified IL-1RA epitopes targeted by anti–IL-1RA antibodies at sites near the IL-1RA/IL-1R interface. Serum from patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) also had elevated levels of anti–IL-1RA autoantibodies compared with those of the controls.

Conclusion: A subset of patients with IgG4-RD have anti–IL-1RA autoantibodies, which promote proinflammatory and profibrotic mediator production via IL-1RA neutralization. These findings support a novel immunologic mechanism underlying the pathogenesis of IgG4-RD. Anti–IL-1RA autoantibodies are also present in a subset of patients with SLE and RA, suggesting a potential common pathway in multiple autoimmune diseases. (J Allergy Clin Immunol 2021;148:715–726)

Key words: IL-1 receptor antagonist, cytokine, IgG4-related disease, lupus, rheumatoid arthritis, autoimmune disease, autoantibodies, plasmablast, sequencing

IgG4-related disease (IgG4-RD) is a fibroinflammatory condition characterized by multiorgan involvement, tissue infiltration by IgG4-expressing plasma cells, and storiform fibrosis.1,2 Autoantibodies against various self-antigens, including a ubiquitin ligase–associated protein,3 carbonic anhydrase IV,4 annexin A11,5 amylase α-2A,6 and galectin-3,7 were previously described in the context of IgG4-RD. Furthermore, passive transfer of purified human IgG and IgG4 antibodies derived from patients with IgG4-RD induced organ manifestations resembling IgG4-RD in recipient mice.8 Nevertheless, the specific autoantibodies that promote inflammation and fibrosis contributing to the diverse organ manifestations in this disease, and their unique targets, are yet to be identified.1 Further, questions remain about the contribution of B cells and autoantibodies to the pathogenesis of IgG4-RD.

Given the broad heterogeneity of organ involvement observed in IgG4-RD, we hypothesized that a subset of autoreactive B cells and autoantibodies target self-antigens that are ubiquitously expressed across multiple inflamed tissues in IgG4-RD. Cytokines are broadly expressed, and anticytokine autoantibodies have been demonstrated in 38% of patients with systemic lupus erythematosus (SLE), 42% of patients with Sjögren syndrome, and 20% of patients with rheumatoid arthritis (RA).9 Here we describe the discovery of a novel target of anticytokine autoantibodies, IL-1 receptor antagonist (IL-1RA), in IgG4-RD, as well as the expression of IL-1RA in the lesional tissues of patients with this disease. We show that anti–IL-1RA autoantibodies can promote expression of inflammatory and fibrotic mediators by neutralizing binding of IL-1RA to the IL-1 receptor (IL-1R). Moreover, we also find elevated levels of anti–IL-1RA autoantibodies in patients with SLE and RA. Together, these findings...
suggest a central mechanism by which anticytokine autoantibodies targeting IL-1RA could promote inflammation and tissue damage in multiple autoimmune conditions.

METHODS

Human subjects

Human samples were collected under protocols approved by the institutional review boards at Stanford University (Stanford, Calif), Massachusetts General Hospital (Boston, Mass), and the University Hospital Heidelberg (Heidelberg, Germany). Patients provided written informed consent. Patients with IgG4-RD (n = 14), patients with RA (n = 55), and patients with SLE (n = 64) were recruited from Stanford Hospital. Samples from patients with multiple sclerosis (MS) (n = 36) were collected from Stanford Hospital and University Hospital Heidelberg. Additional samples from patients with IgG4-RD (n = 147) were collected at Massachusetts General Hospital.

The diagnosis of IgG4-RD was made as described by Umehara et al, and patients were defined as individuals having definite (positive for 3 diagnostic criteria) or probable (positive for criteria 1 and criteria 2 or 3) IgG4-RD. The control groups included age- and sex-matched healthy donors and subjects with other immune-mediated diseases (Sjögren syndrome or gout).

Antibody sequencing, expression, and analysis

Flow cytometry was used to isolate CD19⁺ CD20⁺ CD27⁺ CD38⁻IgG⁺ plasmablasts. As described previously, immunoglobulin heavy chain and light chain genes were cell barcoded, PCR-amplified, and sequenced using MiSeq (Illumina). Bioinformatic analysis was used to assign cell barcodes, obtain error-corrected consensus sequences, and pair the heavy chain and light chain sequences expressed by individual plasmablasts. Clonal family antibodies were identified on the basis of sharing of heavy chain and light chain variable and joining genes and possessing more than 60% CDR3 region identity, as described elsewhere. Recombinant mAbs were expressed and characterized by using antigen microarrays containing 335 cytokines, chemokines, and growth factors.

In vitro blocking assays

A quantity of 40 pg/μL of human IL-1RA (PeproTech) was preincubated with 5 ng/μL of antibody (G4-21 or isotype control). IL-1RA and antibody were applied to HEK-Blue IL-1β cells (InvivoGen) for 2 hours and stimulated with 2 pg/μL of human IL-1β (PeproTech) for 36 hours at 37°C. Alternatively, cells were treated with 8 nM to 10 nM IL-1RA and commercial mAbs or polyclonal antibodies (pAbs) at concentrations of 40 to 2000 nM or with patient plasma at dilutions of 1:10 and 1:20, after which they were stimulated with 0.1 to 1 nM IL-1β for 24 hours. Cells were treated with antibodies in the presence of IL-1β and IL-1RA, IL-1RA alone, or media, and supernatants were assayed by using the QUANTI-Blue assay (InvivoGen; catalog no. hkb-il1b).

Human A549 lung epithelial cells and MRC-5 lung fibroblasts (ATCC) were treated with 10 nM IL-1RA and patient plasma and stimulated with 0.5 nM human IL-1α (PeproTech) in the presence or absence of 1 μM anakinra (Sobi) for 24 hours at 37°C. RNA was isolated, cDNA was prepared, and samples were analyzed by using TaqMan FAM-conjugated primer sets. Supernatants were collected and assayed for human IL-6 (R&D Systems), IL-8 (R&D Systems), and granulocyte-colony-stimulating factor (G-CSF) (R&D Systems) via ELISA.

Immunofluorescence staining

Formalin-fixed, paraffin-embedded tissue sample sections were deparaffined and incubated with diluted primary antibody followed by washing in PBS-Tween and incubation with a secondary antibody and subsequently detected by using the SuperPicTure Polymer Detection Kit (Invitrogen). Images were acquired by using the TissueFAXS platform and quantified with TissueQuest software (TissueGnostics, Austria).

Statistical analysis

Comparisons of IL-1RA reactivity in subjects with 1 of the aforementioned diseases and healthy controls (HCs) were performed by using the Kruskal-Wallis test (>2 group comparisons) or Mann-Whitney U test (2 group comparison). The unpaired Student t test was used for comparison when up to 3 replicates were performed. The Fisher exact test was used for groups with and without detectable levels of anti–IL-1RA autoantibodies in the validation cohort. P values less than .05 were considered statistically significant.

RESULTS

Identification of IL-1RA autoantigen in IgG4-RD

We performed barcode-enabled single-cell sequencing of the heavy and light chain variable regions of IgG plasmablasts from 4 individuals with IgG4-RD. Using bioinformatics analysis, we identified and selected immunoglobulin sequences representative of 42 clonal family plasmablasts (shared heavy chain and light chain variable and joining region gene use and greater than 60% sequence identity in their CDR3 regions) across these 4 patients for recombinant expression as mAbs. Analysis of these mAbs for binding on a protein microarray containing 335 unique human cytokines, chemokines, and growth factors revealed that 1 mAb, G4-21, robustly reacted with IL-1RA cytokine (Fig 1, A and C and see Figs E1 and E2 in this article’s Online Repository at www.jacionline.org). We also observed significantly higher levels of G4-21 reactivity against IL-1RA than the levels of related cytokines, IL-1α, and IL-1β when analyzed via ELISA (Fig 1, D). We did not detect reactivity to IL-1RA in mAbs derived from unrelated plasmablast clonal families from the same patient (G4-5), an additional patient with IgG4-RD (G4-25), or the isotype control mAbs (Fig 1, E).

Anti–IL-1RA autoantibody levels are elevated in patients with IgG4-RD

We evaluated the prevalence of anti–IL-1RA autoantibodies in patients with IgG4-RD by performing ELISA on plasma from patients in a discovery cohort of IgG4-RD (n = 13), disease controls consisting of patients with Sjögren syndrome (n = 6) and gout (n = 6), and HCs (n = 10) (Fig 2, A). We observed significantly higher levels of reactivity to IL-1RA in plasma from patients with IgG4-RD than in the disease controls or HCs (Fig 2, A), with 5 of 13 patients (38%) positive for reactivity against IL-1RA. Findings were confirmed via detection of significantly higher levels of IgG reactivity to IL-1RA in serum from a large, independent cohort of patients with IgG4-RD (n = 122) than in serum from HCs (n = 50) (Fig 2, B and see Table E1 in...
FIG 1. Identification of IL-1RA as a target of a plasmablast-derived IgG antibody in a patient with IgG4-RD. 
A. Phylogenetic tree of the plasmablast antibody repertoire from 1 patient with IgG4-RD. Each peripheral node represents an antibody expressed by a single plasmablast. Red dots indicate clonal family antibodies. (Inset) Magnification of the branch containing the plasmablast-derived mAb G4-21. B. Quantification of the binding reactivity of 42 recombinantly expressed mAbs (derived from the plasmablast clonal families of 4 patients with IgG4-RD) against IL-1RA from the human cytokine microarray analysis. C. Quantification of G4-21 binding to the top 25 reactive antigens on the human cytokine microarray. D and E, ELISA quantification of G4-21 binding to recombinant human IL-1RA, IL-1α, or IL-1β (D) and the additional mAbs G4-05 and G4-25 (E) to IL-1RA. Recombinant human TNF-α and BSA and reactivity of a human IgG secondary antibody alone (E) and an anti–desmoglein 3 (isotype α-DSG3) antibody served as negative controls (B-E). Data represent means ± SDs of the median fluorescent intensities (MFIs) of 4 replicate spots of each antigen (B and C) or europium counts of triplicate wells (D and E). ***P < .001 by 2-tailed unpaired Student t tests.
Elevation in levels of circulating IgG4 antibodies is observed in 90% of patients with IgG4-RD. Thus, we evaluated the IgG subclasses of circulating anti–IL-1RA autoantibodies of the patients.
with IgG4-RD in our discovery cohort. IL-1RA reactivity was detected via ELISA for each IgG subclass (IgG1-IgG4) in plasma from the patients with IgG4-RD (Fig 2, C). We observed significantly higher IgG4 reactivity against IL-1RA in patients with IgG4-RD (n = 14; an additional patient was recruited to the discovery cohort at the time of this experiment) than in the HCs (n = 10) (Fig 2, C). Interestingly, 14.2% of the samples from patients with IgG4-RD from our validation cohort were positive for IgG4 anti–IL-1RA autoantibodies, representing more than 90% of the patients who were also positive for total IgG anti–IL-1RA (Fig 2, D). Levels of IgG1- and IgG3-specific reactivity to IL-1RA were also significantly higher in patients with IgG4-RD than in HCs. These results indicate that IgG4 is the predominant subclass in the autoantibody response toward human IL-1RA, although IL-1RA–specific IgG1, IgG2, and IgG3 antibodies are also present in a subset of patients with IgG4-RD.

Anti–IL-1RA antibodies promote IL-1–mediated expression and production of inflammatory and fibrotic mediators

We investigated whether anti–IL-1RA autoantibodies mediate inflammatory and fibrotic processes in IgG4-RD by antagonizing IL-1RA using the HEK-Blue IL-1β reporter line in which IL-1R activity is measured through the detection of secreted embryonic alkaline phosphatase (SEAP) (see Fig E3 in this article’s Online Repository at www.jacionline.org). Incubating IL-1RA/IL-1β–stimulated reporter cells with the patient-derived mAb G4-21 resulted in a significant increase in SEAP production as compared with that in the control antibodies (Fig 3, A). Significant increases in SEAP production were also observed in a dose-dependent manner when reporter cells were incubated with increasing concentrations of the commercial anti–IL-1RA pAb 200-01RA as compared with when a pAb isotype control was used (Fig 3, B). Moreover, reporter cells produced significantly more SEAP when incubated with plasma from patients with IgG4-RD (n = 13), with 54% of them positive for neutralizing anti–IL-1RA autoantibodies than when incubated with plasma from HCs (n = 15) (Fig 3, C). Together, these findings indicate that both recombinantly expressed antibodies and antibodies present in plasma from patients with IgG4-RD can antagonize the function of IL-1RA.

We investigated whether anti–IL-1RA antibodies could abrogate the regulatory activity of IL-1RA in epithelial and fibroblast cell lines stimulated with IL-1α and promote inflammatory and fibrotic responses. We measured expression of genes encoding multiple inflammatory cytokines (IL-1α, IL-6, IL-8, IL-33, TNF-α, and G-CSF), growth factors (PDGF-α, PDGF-b, PDGF-c, and CTGF), extracellular matrix proteins (MMP-3, MMP-7, MMP-9, MMP-12, MMP-13, and TIMP1), and collagen synthesis proteins (COL1A and COL4A) in A549 epithelial cells and MRC5 fibroblasts stimulated with IL-1α and IL-1RA in the presence or absence of the pAb 200-01RA. Treatment of IL-1α–stimulated A549 and MRC5 cells with IL-1RA resulted in significant changes in the mRNA levels of these genes compared with IL-1α–stimulated cells alone (Fig 3, D and see Figs E4, A-D and E5, A in this article’s Online Repository at www.jacionline.org). Addition of the pAb 200-01RA to IL-1α/IL-1RA–stimulated A549 and MRC5 cells also significantly altered expression of these genes, resembling conditions with IL-1α stimulation alone. (Fig 3, D and see Figs E4 and E5, A). Further, IL-6 and IL-8 protein levels were significantly elevated in the supernatants of both IL-1α/IL-1RA–stimulated A549 and MRC5 cells following treatment with the pAb 200-01RA as compared with IL-1α/IL-1RA alone (Fig 3, E and see Fig E5, B).

We investigated whether anti–IL-1RA autoantibodies in the plasma of patients with IgG4-RD could promote expression of these proinflammatory and profibrotic mediators in vitro. We treated IL-1α/IL-1RA–stimulated A549 or MRC5 cells with plasma from 3 patients with IgG4-RD in our discovery cohort that demonstrated reactivity to IL-1RA. Plasma from each patient partially restored IL-6, IL-33, and MMP-9 (for A549 cells) and IL-6, IL-8, and G-CSF (for MRC5 cells) mRNA levels to those observed in the IL-1α–stimulated cells (Fig 3, F and see Fig E5, C). Additionally, IL-6, IL-8, and G-CSF protein levels were significantly elevated in the supernatants of A549 and MRC5 cells treated with plasma of patients with IgG4-RD for 24 hours (Fig 3, G and see Fig E5, D). These increases were not attributed to endogenous IL-1 present in the plasma samples (see Fig E5, E).

Together, these findings indicate that both recombinant and IgG4-RD patient plasma–derived anti–IL-1RA autoantibodies can directly modulate the expression of key mediators involved in inflammation, tissue remodeling, and fibrosis through the antagonism of IL-1RA in vitro.

Anti–IL-1RA autoantibodies are linked to the number of affected organs in IgG4-RD, and IL-1RA is expressed in lesional tissues

We explored the relationship between organ involvement and anti–IL-1RA autoantibodies by quantifying the number of organs involved in patients with IgG4-RD with or without IgG4 anti–IL-1RA autoantibodies (Fig 4, A). On average, patients with anti–IL-1RA autoantibodies had greater numbers of organs involved than did those who were negative for anti–IL-1RA autoantibodies (Fig 4, A), suggesting that anti–IL-1RA antibodies correlate to multorgan manifestations of IgG4-RD.

Overexpression of self-proteins in the tumefactive lesions of patients with IgG4-RD is associated with breaches of humoral immune responses, including the presence of autoantibodies. We examined expression of IL-1RA in patients with IgG4-RD by performing multicolor immunofluorescence in the lesional tissues from 4 patients with IgG4-RD. IL-1RA was present in the kidney, lacrimal gland, lymph node, and salivary gland tissues from these patients (Fig 4, B). Quantification indicated that there were significantly more IL-1RA–positive cells in salivary gland tissues affected by IgG4-RD than in a control sample from a patient with Sjögren syndrome (Fig 4, C). Although our tissue sample size was small, these findings demonstrate that IL-1RA, the target of anti–IL-1RA autoantibodies, is present at sites of tissue damage in IgG4-RD.

Peptide epitope mapping of anti–IL-1RA antibodies

Given that multiple anti–IL-1RA antibodies were able to inhibit IL-1RA function, we mapped the binding sites of these antibodies on IL-1RA. We tested the binding of multiple anti–IL-1RA antibodies to 16 overlapping 19-mer peptides spanning the IL-1RA protein by ELISA (see Fig E6 in this article’s Online Repository at www.jacionline.org). The mAb G4-21 exhibited increased binding to the peptide RA-05 as compared with the human IgG isotype control mAb (Fig 5, A). The
A commercial anti-human/murine IL-1RA mAb MAB4801 demonstrated significantly increased binding to RA-12, with more than a 15-fold change in binding reactivity over that demonstrated by a rat IgG1 isotype control mAb (Fig 5, B). In addition, the pAb 200-01RA showed significantly increased binding to all IL-1RA peptides, with the highest levels of reactivity (>50-fold change) to the peptides RA-04, RA-05, and RA-06 (cluster 1); RA-08, RA-09, and RA-10 (cluster 2); RA-12 (cluster 3); and RA-14, RA-15, and RA-16 (cluster 4) compared with the levels in a pAb isotype control (Fig 5, C). A comparison of the levels of peptide reactivity across all 3 anti-IL-1RA antibodies revealed 2 shared peptide epitopes in

FIG 3. Anti–IL-1RA antibodies exacerbate IL-1–induced expression of inflammatory and fibrotic mediators and are inhibited by anakinra. A-C, Quantification of SEAP produced by HEK-Blue IL-1β reporter cells incubated with human IL-1β and IL-1RA in the presence of the mAb G4-21 or control antibodies (A); a commercial anti–IL-1RA pAb at 40, 80, 160, and 320 nM (B); or plasma from patients with IgG4-RD (n = 13) or HCs (n = 15) (C). Threshold for IL-1RA neutralization (3 SDs above the mean OD for the HC group) is indicated by a green line (C). D-G, Quantification of mRNA (D and F) and protein levels (E and G) of inflammation- and fibrosis-associated genes in IL-1α–stimulated A549 epithelial cells incubated with an anti–IL-1RA pAb at 100 nM (D) and at 50, 100, and 200 nM (E) or plasma from 3 patients with IgG4-RD who were positive for anti–IL-1RA autoantibodies (F and G) in the presence of IL-1RA. H, ELISA quantification of IL-6, IL-8, and G-CSF supernatant levels in IL-1α–stimulated A549 cells incubated with plasma from patients with IgG4-RD with IL-1RA and anakinra at 1 μM. Data represent means ± SDs of at least 2 wells and at least 2 independent experiments. *P < .05; **P < .01; and ***P < .001 by the Kruskal-Wallis test (A), Mann-Whitney U test (B and C), or the 2-tailed unpaired Student t test (D-H).
clusters 1 and 3 (i.e., RA-05 and RA-12) that bound each IL-1RA-specific mAb and the pAb 200-01RA and 2 unique peptide epitopes representative of clusters 2 and 4 (i.e., RA-09 and RA-16) that are uniquely targeted by 200-01RA.

We evaluated the prevalence of levels of reactivity against the 4 specific nonoverlapping IL-1RA peptides (i.e., RA-05, RA-09, RA-12, and RA-16) in plasma from our discovery cohort of patients with IgG4-RD (n = 13). Three patients with IgG4-RD had positive reactivity against at least 1 peptide epitope, demonstrating that multiple regions of IL-1RA may be targeted by circulating IgG autoantibodies in patients with IgG4-RD (Fig 5, D).

Mapping of these peptides onto a previously determined crystal structure of the IL-1RA/IL-1R complex (PDB:1IRA)16 demonstrated that these anti–IL-1RA antibodies target epitopes at or proximal to (RA-05 and RA-12) and distal to (RA-09 and RA-16) in plasma from our discovery cohort of patients with IgG4-RD (n = 13). Three patients with IgG4-RD had positive reactivity against at least 1 peptide epitope, demonstrating that multiple regions of IL-1RA may be targeted by circulating IgG autoantibodies in patients with IgG4-RD (Fig 5, D).

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Anti–IL-1RA autoantibodies exhibit cross-reactivity to other IL-1 family members

On the basis of high degrees of homology, we tested whether anti–IL-1RA autoantibodies cross-react with IL-36RA or IL-38. We observed significantly higher levels of reactivity to IL-36RA and IL-38 with mAb G4-21 and the pAb 200-01RA than with the TGF-α and BSA controls (Fig 6, A and B). We did not detect reactivity to IL-36RA or IL-38 in mAbs derived from unrelated plasmablast clonal families (G4-05 and G4-25), or the isotype control mAb (Fig 6, A). Alignment of the sequences of the IL-1RA peptides RA-05, RA-09, RA-12 and RA-16 with the corresponding regions on IL-36RA and IL-38 revealed high sequence homology and topology across the 3 receptor antagonist cytokines (Fig 6, C). These findings highlight potential peptide epitopes by which IL-1RA-targeting autoantibodies cross-bind IL-36RA and IL-38.

Anti–IL-1RA autoantibodies detected in patients with SLE and RA

Compared with healthy subjects, patients with SLE have elevated levels of IgG reactivity to cytokines that correlate with disease severity.14 Therefore, we investigated the presence of anti–IL-1RA autoantibodies in serum samples from a cohort of patients with SLE. Serum from patients with SLE (n = 49) contained significantly higher levels of reactivity against IL-1RA than did serum from HCs (n = 25), with 16 of 49 patients with SLE (33%) positive for anti–IL-1RA autoantibodies (Fig 7, A). We also analyzed IL-1RA reactivity in the serum of patients with either MS (n = 36) or RA (n = 55). Similar in patients with SLE, levels of IL-1RA reactivity were significantly elevated in 25% of patients with RA as compared with in the HCs (n = 50), but not in patients with MS (Fig 7, B). These results indicate that anti–IL-1RA autoantibodies are
FIG 5. Anti–IL-1RA antibodies target IL-1RA peptides near the IL-1R. A-C, ELISA quantification of the mAb G4-21 (derived from a patient with IgG4-RD) (A), the commercial anti–IL-1RA mAb, MAB4801 (B), and the commercial anti–IL-1RA pAb 200-01RA (C) binding to 16 overlapping 19-mer human IL-1RA peptides. Fold change represents europium counts of each peptide over the minimum peptide reactivity (across all peptides) per antibody. D, ELISA quantification of IgG binding to 4 immunogenic IL-1RA peptides identified in (A-C) in the plasma of patients with IgG4-RD (n = 13) and HCs (n = 15). Data represent means ± SDs of the europium counts of triplicate wells. Green line indicates the threshold for IL-1RA peptide binding (3 SDs above the mean europium count from the HC group). Colored data points indicate samples from patients with IgG4-RD that were positive for autoantibodies for a given peptide. E, Three-dimensional structure of human IL-1R (blue) in complex with human IL-1RA (Protein Data Bank no. 1IRA) (green). (Left) IL-1RA residues implicated in binding IL-1 Receptor at domains I and II and at their interface are highlighted in red, purple, and orange, respectively. (Right) Peptides 5, 9, 12, and 16 targeted by anti–IL-1RA autoantibodies are highlighted in red. ***P < .001 by 2-tailed unpaired Student t tests.
detectable in subsets of patients with SLE and RA and that antagonism of IL-1RA–mediated responses could contribute to inflammation in these autoimmune diseases and IgG4-RD.

DISCUSSION

Autoantibodies targeting cytokines are present in several autoimmune and inflammatory diseases, and they can be pathogenic by promoting proinflammatory responses. Although multiple putative antigen targets of autoreactive IgG antibodies have been identified in IgG4-RD, whether and how these and other antibodies contribute to the fibroinflammatory nature and diverse organ manifestations observed in this disease remain unclear. Here we have identified neutralizing anti–IL-1RA autoantibodies in IgG4-RD and characterized their role in promoting the expression of proinflammatory and profibrotic mediators via antagonism of IL-1RA function. We have also shown that similar antibodies are present in the sera of patients with SLE and RA. Together our findings uncover a potential common mechanism for the promotion of inflammatory and fibrotic pathways that could contribute to the pathophysiology in these diseases.

We have demonstrated that plasma autoantibodies from patients with IgG4-RD can promote expression of proinflammatory and profibrotic factors, including MMP-9 and IL-33, in epithelial and fibroblast cell lines stimulated with IL-1α and IL-1RA. These cytokines, enzymes, and growth factors coordinate protective immune responses, including inflammatory responses triggered by infection and wound healing. However, dysregulation of these factors can promote pathogenic inflammation and fibrosis, causing irreversible tissue damage and dysfunction. Activation of TGF-β1 and MMP9 via overexpression of IL-13 can promote airway fibrosis in mice, similar to that observed in chronic kidney disease. IL-33, an IL-1 family inflammatory cytokine, can also promote lung fibrosis in bleomycin-treated mice through regulation of MMP-9, TGF-β1, and other fibrotic mediators. Together, these findings support the possibility that anti–IL-1RA autoantibodies can drive inflammation and fibrosis in IgG4-RD and other autoimmune diseases by promoting expression of these factors as a result of blocking IL-1RA activity.

We have identified 4 IL-1RA immunogenic peptide epitope targets of monoclonal and polyclonal anti–IL-1RA antibodies and autoantibodies from patients with IgG4-RD. Anti–IL-1RA antibodies targeting peptide epitopes at the IL-1RA/IL-1R interface may sterically block this interaction, whereas anti–IL-1RA antibodies that target peptide epitopes outside of this interaction could induce conformational changes that weaken the affinity of IL-1RA for IL-1R. Neutralizing anti–IL-1RA antibodies that
target these epitopes and disrupt the IL-1RA/IL-1R interaction may thereby allow IL-1R to bind IL-1 and promote signaling. Furthermore, in vitro cross-reactivity between anti–IL-1RA antibodies and the functionally related cytokines IL-36RA and IL-38, which inhibit IL-36 signaling, suggests that the mechanism through which anti–IL-1RA antibodies promote inflammatory and fibrotic mediator production may be broader than through targeting of IL-1RA alone. Future studies will need to investigate the role of IL-36 in the pathogenesis of IgG4-RD, as well as whether abrogation of IL-36RA and IL-38 function by autoantibodies plays a significant role in this disease.

IgG4 antibodies were the predominant subclass of anti–IL-1RA reactivity that we identified, and they are a diagnostic feature of IgG4-RD.15 IgG4 molecules typically exhibit anti-inflammatory properties; however, they have been shown to promote disease symptoms in an Fc-independent manner in pemphigus vulgaris and myasthenia gravis through binding to skin- and muscle-specific autoantigens.29-31 As we have identified epitopes suggesting that anti–IL-1RA antibodies disrupt the IL-1RA/IL-1R interaction, it is possible that a subset of antibodies in IgG4-RD promotes pathogenesis independently of Fc signaling or complement activation. We have also identified reactivity against IL-1RA in all IgG subclasses in patients with IgG4-RD, suggesting that antibodies of other IgG subclasses (IgG1-IgG3) may contribute to disease pathogenesis via engagement of their Fc regions.

Given the rationale for contributions of all IgG subclasses to disease, autoantibody-driven pathogenesis in IgG4-RD may occur through multiple mechanisms. We recently demonstrated that tissue-infiltrating B cells in IgG4-RD autoimmune pancreatitis produce profibrotic mediators.32 As we have detected IL-1RA in the tissues of affected organs in IgG4-RD, activated B cells in these tissues may, in part, target IL-1RA and promote tissue inflammation and fibrosis via overexpression and secretion of inflammatory and fibrotic mediators.

This study has several limitations. First, although large for a rare disease such as IgG4-RD, our patient cohort was small, and depth of sequencing may have limited our ability to identify additional key autoantibodies. Nevertheless, we also identified an additional autoantigen target, macrophage inflammatory protein 3 (MIP3a [an alias symbol of CCL20]), in our cytokine microarray analysis of plasmablast-derived mAbs and plasma samples from IgG4-RD. Further studies are needed to elucidate its role in IgG4-RD. Second, although we identified peptide epitopes for anti–IL-1RA autoantibodies that structurally implicate disruption of the IL-1RA/IL-1R interaction, further studies are needed to discern the mechanisms by which anti–IL-1RA antibodies inhibit IL-1RA function and determine whether these autoantibodies mediate pathogenesis in an Fc-dependent or Fc-independent manner. Finally, additional studies are needed to characterize the roles of anti–IL-1RA autoantibodies in SLE and RA, as well as to determine whether these autoantibodies represent a common pathogenic mechanism.

In conclusion, we have described the identification of anti–IL-1RA autoantibodies in patients with IgG4-RD that antagonize the inhibitory effects of IL-1RA, thereby promoting the production of proinflammatory and profibrotic mediators implicated in disease pathogenesis. Identification of IL-1RA in IgG4-RD tissue lesions, as well as association of multiorgan involvement in individuals with anti–IL-1RA autoantibodies of the IgG4 subclass, suggests an underlying molecular mechanism for the varied organ manifestations in this disease. The cross talk between anti–IL-1RA antibodies and other IL-1RA family members and the presence of these autoantibodies in SLE and RA suggest a broader function for autoantibodies.
targeting IL-1RA family members and an underappreciated mechanism for the pathogenesis of multiple autoimmune diseases. Future studies will be critical for evaluating the diagnostic, prognostic, and pathogenic potential of anti–IL-1RA autoantibodies in IgG4-RD, as well as for addressing whether inhibition of IL-1 may be an advantageous route for the treatment of anti–IL-1RA–driven disease.

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Key messages
- Anti–IL-1RA autoantibodies are present in a subset of patients with IgG4-RD and are correlated with the number of affected organs.
- Anti–IL-1RA antibodies promote the production of proinflammatory and profibrotic mediators by abrogating the activity of IL-1RA.
- Anti–IL-1RA antibodies bind to IL-1RA at sites proximal and distal to the IL-1RA/IL-1R interface, likely modulating interactions between IL-1RA and IL-1R.

REFERENCES