







SHORT REPORT

Anticitrullinated peptide antibody epitope expansion and the HLA DRB1 'shared epitope' are less common in seropositive checkpoint inhibitor-induced inflammatory arthritis than in longstanding rheumatoid arthritis

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ABSTRACT

Background Immune checkpoint inhibitors (ICI) can potentially cause ICI-inflammatory arthritis (ICI-IA), which often resembles rheumatoid arthritis (RA). In this study, we examined the degree of anticitrullinated peptide antibodies (ACPA) epitope expansion in CCP+ICI-IA and patients with RA.

Methods We used clinical data and serum from ICI-IA and patients with RA with early disease as well as longstanding disease. A custom, bead-based antigen array was used to identify IgG ACPA reactivities to 18 putative RA-associated citrullinated proteins. Hierarchical clustering software was used to create a heatmap to identify ACPA levels. Additionally, HLA DRB1 typing was performed on ICI-IA patients as well as controls of patients treated with ICI that did not develop ICI-IA (ICI controls).

Results Compared to patients with CCP+RA, patients with CCP+ICI-IA were older ($p<0.001$), less likely to have positive rheumatoid factor ($p<0.001$) and had a shorter duration of symptoms ($p<0.001$). There were less ACPA levels and a lower number of distinct ACPA epitopes in the serum of patients with ICI-IA compared with longstanding patients with RA ($p<0.001$). Among those tested for HLA DRB1, there were no differences in the frequency of the shared epitope between those with ICI-IA and ICI controls.

Conclusion Patients with ICI-IA had lower ACPA titres and targeted fewer ACPA epitopes than longstanding patients with RA, and there were no significant differences in the presence of the shared epitope between those that developed ICI-IA and ICI controls. It remains to be determined if ICI-IA represents an accelerated model of RA pathogenesis with ICI triggering a transition from preclinical to clinical disease.

WHAT IS ALREADY KNOWN ON THE TOPIC

⇒ immune checkpoint inhibitor (ICI)-inflammatory arthritis (ICI-IA) can clinically resemble rheumatoid arthritis (RA) and some patients with ICI-IA are anticitrullinated peptide antibodies (ACPA) positive.

WHAT THIS STUDY ADDS

⇒ Both patients with ICI-IA and patients with early RA have less ACPA epitope expansion than patients with longstanding RA; however, ICI-IA does not appear to be associated with the HLA DR shared epitope.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ ICI-IA could serve as a model of early seronegative RA with a known trigger.

INTRODUCTION

Immune checkpoint inhibitor-induced inflammatory arthritis (ICI-IA) is a rheumatic complication that occurs in ~4% of ICI-treated patients with cancer.¹ The median time to onset is roughly 12 weeks²; however, some patients present within days of the first dose of ICI, while others can present even after ICI has been discontinued.³ Clinically, ICI-IA resembles rheumatoid arthritis (RA) in over 50% of cases² with a symmetrical polyarthritis involving both small and large joints. Fewer patients with ICI-IA are anti-cyclic citrullinated peptide (CCP) positive than patients with RA, 9% vs 50%,^{2,4} although this is higher than in the general population

where anti-CCP positivity is 0.6%.⁴ The commercially available anti-CCP2 assay is an ELISA that is highly sensitive and specific for RA.⁵ However, antibodies to other citrullinated peptides, such as vimentin, filaggrin and fibrinogen, have also been implicated in patients with RA.⁶ Furthermore, the number of anticitrullinated peptide antibodies (ACPA) increases dramatically in the 2–3 years prior to RA symptom onset, possibly due to epitope expansion.⁷ Another important marker associated with RA is the HLA-DRB1 shared epitope (SE). SE-homozygosity is more prevalent in patients with RA than in healthy controls,^{8,9} and those with the SE are more likely to be ACPA positive than those without (76% vs 45%).¹⁰ We postulate that clinical disease will be triggered at an early time point in RA-susceptible individuals, such as those who are ACPA positive and/or have the SE, when exposed to ICI. In such a scenario, we would expect to find less ACPA epitope expansion in ICI-IA than de novo RA. To test this hypothesis, we compared ACPA reactivities in patients with ICI-IA to patients with early and with long-standing RA. We also examined the prevalence of the SE allele in patients with ICI-IA and ICI-treated patients without arthritis.

METHODS

ACPA analysis

Patients

ACPA testing was performed on patient samples drawn from three prospective cohorts: (1) a single institutional ICI-IA registry (Hospital for Special Surgery) at time of enrolment, (2) the multicentre Canadian Early Arthritis Cohort in the USA (CATCH-US) featuring newly diagnosed patients with RA with less than 1 year of symptoms and (3) a single institutional registry of longstanding patients with RA enrolled at time of arthroplasty (FLARE, Hospital for Special Surgery). All cohorts were approved by the HSS institutional review board, and all patients consented to enrolment and providing both serum and synovial fluid, if available. Serum samples were collected at time of enrolment into the registries, and synovial fluid was collected when available. Anti-CCP screening was done using a commercial ELISA (anti-CCP2 assay, positive >20 units/mL). Clinical characteristics were summarised and compared using Fisher's exact or Kruskal-Wallis test and adjusted for multiple comparisons if warranted.

ACPA assay

A custom, bead-based antigen array was used to identify antibody reactivities to 18 putative RA-associated citrullinated proteins with 51 peptides. In this methodology, antigen-coated MagPlex-Avidin beads are incubated with serum samples followed by conjugated antihuman IgG antibodies, which are then analysed on a Luminex FLEXMAP3D Instrument to determine fluorescence and bead identity. Patient samples were analysed for IgG, IgM and IgA ACPAs. Hierarchical clustering software is then used to create heatmaps to identify ACPAs and signal

intensities representing antibody levels. This microarray has been validated and reproduced against positive and negative controls among patients with RA.⁷ Intercohort signal intensity differences were analysed using Wilcoxon rank-sum testing. The assay was performed on synovial fluid when available.

HLA DRB1 analysis

Patients

HLA DRB1 typing was performed on patients with ICI-IA enrolled in one of two registries, one at Hospital for Special Surgery in New York and one at University of Chicago Medical Center. HLA DRB1 typing was also performed on ICI-treated patients enrolled in the University of Chicago registry who did not develop inflammatory arthritis, who served as ICI-treated controls. Only Caucasian patients were included because the association between the SE and RA is strongest in that population.⁹ The following genotypes were considered SE alleles: HLADRB1*01:01, 01:02, 04:01, 04:04, 04:05, 04:08, 10:01, 14:02.¹¹ Patients with at least one of these alleles (heterozygous) were designated as having the SE. HLA-DRB1 SE frequencies were compared between the patients with ICI-IA and ICI-treated controls.

HLA-DRB1 genotyping

HLA-DRB1 genotyping was performed using single-molecule real-time (SMRT) sequencing performed at the Mount Sinai (New York, New York) core laboratory. GenDx primers that target the entire DRB1 gene locus are used, and full-length amplicons are sequenced on PacBio's SMRT sequencing platform for high-resolution phasing of both alleles for each sample

RESULTS

ACPA

A total of 75 CCP-positive patients were included in the ACPA analysis: 12 patients with ICI-IA, 39 patients with early RA (CATCH-US) and 24 patients with late RA (FLARE). Demographics and clinical characteristics are summarised in [table 1](#). None of the ICI-IA patients with imaging reports (8) had erosive disease; maximum Clinical Disease Activity Index (CDAI) ranged from 5 to 32.5 (median 11.3). Disease duration was a median of 3.7 months (1.0, 11.3) in ICI-IA, 6.7 months (4.0, 9.7) in CATCH-US and 232 (184, 376) months in FLARE ($p<0.001$). Patients with ICI-IA were older ($p<0.001$) and trended towards being less predominantly women ($p=0.08$). Fewer patients with ICI-IA were RF (IgM) positive ($p=0.002$) and there was a trend towards their having lower anti-CCP titres ($p=0.06$). Sixty-seven percent of CCP-positive patients with ICI-IA were current or past smokers compared with 38% in the early RA cohort and 54% in the late RA cohort.

A heatmap demonstrating ACPA IgG reactivities is shown in [figure 1](#). Patients with ICI-IA had overall lower IgG ACPA antibody levels than patients with late RA ($p<0.01$), and patients with early RA had lower IgG

Table 1 Baseline characteristics of CCP+ICI-IA, CATCH-US and FLARE patients

	ICI-IA (N=12)	CATCH-US (N=39)	FLARE (N=24)	P value
Age in years, mean (SD)	71.0 (8.3)	48.2 (14.6)	64.27 (10.9)	p<0.001
Female sex	7 (58%)	33 (85%)	22 (92%)	0.08
White/Caucasian	9 (75%)	27 (69%)	19 (79%)	0.8
Symptom duration in months, median (IQR)	3.7(1.0,11.3)	6.7(4.0,9.7)	232(184,376)	p<0.001
RF positive	1 (8%)	27 (71%)	13 (54%)	p=0.002
CCP level (units/mL), median (IQR)	42.2 (29.4,70.5)	250 (107.5,251.0)	239 (28.5,251.0)	0.06
Obese (BMI≥30)	3 (25%)	9 (23%)	7 (29%)	0.2
Current/past smoker	8 (67%)	14 (38%)	13 (54%)	0.2

Bolded values are statistically significant.
 .BMI, body mass index; CATCH, Early RA cohort; CCP, cyclic citrullinated peptide; FLARE, Late RA cohort; ICI-IA, immune checkpoint inhibitor inflammatory arthritis; RF, rheumatoid factor.

signal reactivity than the patients with late RA ($p<0.001$). Patients with ICI-IA had slightly higher ACPA IgG reactivity than early RA ($p=0.007$) but after removing the one ICI-IA patient that was an outlier with reactivity to almost every ACPA epitope, the ICI-IA group was not statistically different than the early RA group ($p=0.9$). ICI-IA also exhibiting significantly much less IgM reactivity than either RA group ($p<0.001$, data not shown). As a whole, patients with ICI-IA had antibodies to fewer ACPA epitopes than patients with RA, though there was one ICI-IA patient who reacted to almost every epitope. The synovial fluid analysis did not demonstrate any significant ACPA reactivity using this specific assay (data not shown).

HLA-DRB1 genotyping

HLA-DRB1 genotyping was performed in 45 patients with ICI-IA from New York, 20 patients with ICI-IA from Chicago and 119 controls, or ICI-treated patients without arthritis, from Chicago (table 2). Age, sex, ICI regimen and smoking status were similar between the groups.

More patients from Chicago had melanoma than from New York. The per cent of patients who were CCP positive was similar in the two ICI-IA groups. Forty-three per cent of ICI-IA and 46% of ICI-treated controls had at least one SE allele. SE heterozygosity was 31% in patients with ICI-IA and 39% in controls. SE homozygosity was 12% in patients with ICI-IA and 8% in controls ($p=0.4$). Of the 65 patients with ICI-IA, only 6 (9%) were positive for either DRB1-01 or DRB1-04 alleles. Only seven CCP-positive patients with ICI-IA could be HLA DRB1 genotyped; two had the SE (one homozygous DRB1-04, one heterozygous DRB1-04/DRB1-11), both past smokers.

DISCUSSION

In this study, we demonstrate that seropositive patients with ICI-IA have lower ACPA levels and target fewer ACPA epitopes than patients with established RA. Our results may suggest that ICI treatment jump-starts arthritis onset in ACPA-positive patients with cancer or, alternatively,

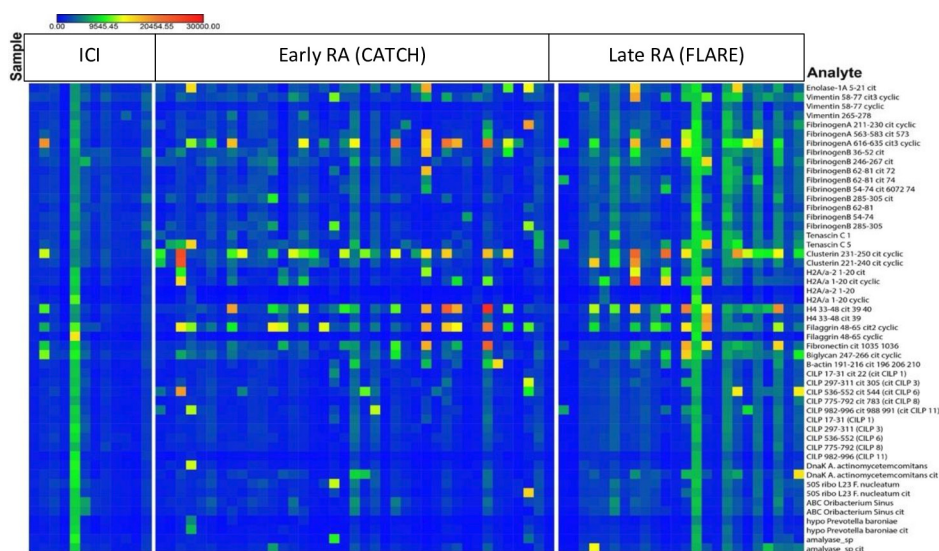

Figure 1 IgG anticitrullinated peptide antibody (ACPA) reactivities for immune checkpoint inhibitor (ICI) cohort, early RA (CATCH) cohort and longstanding RA (FLARE) cohort; ACPA epitopes listed along the side.

Table 2 Baseline characteristics of patients with ICI-IA and of ICI-treated controls without arthritis with available HLA DRB1 testing

	ICI-IA (NY, n=45)	ICI-IA (IL, n=20)	ICI-IA pooled (NY+IL, n=65)	ICI controls (IL, n=119)	P value (pooled vs controls)
Age in years, mean (SD)	69.8 (11.9)	61.0 (11.6)	67.1 (11.8)	63.7 (13.5)	0.1
Female sex, n (%)	9 (20)	10 (50)	19 (29)	32 (27)	0.7
Cancer type, n (%)					p=0.004
Melanoma	9 (20)	10 (50)	19 (29)	59 (50)	
NSCLC	8 (18)	1 (5)	9 (14)	3 (3)	
Urothelial/RCC	16 (36)	1 (5)	17 (26)	25 (21)	
Other	12 (26)	8 (40)	20 (31)	32 (27)	
ICI regimen, n (%)					0.4
PD-1/PD-L1	33 (73)	13 (65)	46 (71)	74 (62)	
CTLA-4	0 (0)	0 (0)	0 (0)	3 (3)	
Combination	12 (27)	7 (35)	19 (29)	41 (35)	
Current/past smoker, n (%)	26 (58)	11 (50)	37 (60)	69 (58)	0.9
CCP positivity, n (%)	5 (11)	2 (10)	7 (11)	--	--
SE positivity, n (%)	19 (42)	9 (45)	28 (43)	55 (46)	0.8
Homozygous, n (%)	5 (11)	3 (15)	8 (12)	9 (8)	0.4

.CCP, cyclic citrullinated peptide; ICI-IA, immune checkpoint inhibitor inflammatory arthritis; IL, Illinois; NSCLC, non-small cell lung cancer; NY, New York; RCC, renal cell carcinoma; SE, shared epitope.

that ACPA reactivity develops in parallel with arthritis symptoms after ICI initiation. Although we did not have pretreatment serum samples on our patients with ICI-IA to help distinguish these two possibilities, Belkhir *et al* reported that anti-CCP was present prior to ICI treatment in two of the three CCP-positive patients with ICI-IA they had tested.¹² ACPA reactivity was heterogenous among the patients with ICI-IA in this study. One patient, for example, had reactivity to almost every citrullinated peptide tested. We could not find any clinical characteristics to distinguish this patient from the others. However, this patient as well as the ICI-IA patient with the second most IgG reactivity had the highest plasma CCP titres of the group (>250 units/mL). And, thus, while we cannot comment on the affinity-avidity of IgG in this assay, we can conclude that ACPA epitope reactivity is reflective of plasma CCP titres.

We did not find a higher prevalence of the RA-associated HLA DR 'SE' in patients with ICI-IA patients, whether seropositive or negative, compared with ICI-treated patients without arthritis. This observation differs from Cappelli *et al*,¹³ who demonstrated a higher frequency of SE heterozygosity in patients with ICI-IA, though that was when compared with healthy blood donors. The SE is a five amino acid sequence in the peptide binding groove of the HLA DRB1 molecule.⁸ SE-homozygosity is four times more prevalent in patients with RA than in healthy controls, and patients with RA with the SE are more likely to be ACPA positive than those without.¹⁰ Smoking has also been implicated as a risk factor for the development of RA, and the association of the SE with

RA is only found in ACPA-positive patients with a history of smoking.⁹ Furthermore, it has been suggested that the SE and smoking act at different stages of arthritis development; smoking confers risk for ACPA and symptom development, whereas SE mediates arthritis development.¹⁴ Although over half of our cohort were current/past smokers, we had too few CCP-positive patients to assess the relationship between anti-CCP, smoking and the SE in this population. The number of patients with only DRB1-01 or DRB1-04 alleles, which are specifically associated with ACPA positivity, was also too few to find meaningful associations.

While we hypothesised that ICI-IA may be a preclinical model for RA, the lack of SE association as well as limited ACPA seropositivity suggests otherwise. Perhaps ICI-IA is more akin to seronegative RA, where genetic polymorphisms in the interferon (IFN) pathway have been implicated.¹⁵ A recent study of synovial fluid CD8+T cells from patients with ICI-IA demonstrated a strong IFN signature, a signature that was much stronger than was seen in patients with RA or PsA.¹⁶ In summary, patients with ICI-IA were found to have fewer ACPA epitopes and lower ACPA concentrations than patients with longstanding RA. Longitudinal assessment of patients with cancer beginning prior to ICI initiation up through the development of ICI-IA, in addition to histopathological assessment of synovium from patients with ICI-IA, could provide more insight into whether ICI-IA shares characteristics with seronegative RA, or it is an entirely different entity.

Contributors NG was responsible for study conception, data collection, analysis, manuscript preparation. PR was responsible for providing patient data, data collection, manuscript preparation. CAA was responsible for data collection, analysis, manuscript preparation. JK was responsible for data analysis, manuscript preparation. SMG was responsible for providing patient data, manuscript preparation. VPB was responsible for providing patient data, manuscript preparation. AL was responsible for sample preparation and manuscript preparation. DR was responsible for data collection, manuscript preparation. KKC was responsible for study conception, providing patient data, manuscript preparation. WHR was responsible for data analysis, manuscript preparation. ARB was responsible for study conception, providing patient data, data collection, analysis, manuscript preparation.

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Competing interests SG has received grants from Novartis and is on an ACR guideline subcommittee. NG has received an institutional grant and is on an ACR guideline subcommittee. ARB has received grants from her institutions and the RRF, is on the ACR Board of Directors, ACR guideline subcommittee and chair of the Committee of Ethics. VB has participated in institutional grants and/or received consulting fees from Amgen, Abbvie, BMS, Janssen, Genzyme, Regeneron, Gilead, Pfizer, UCB, Cedar Hill Foundation, NIH and Novartis. WB has received grants from NIH.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by HSS IRB—2017-1898, 19-08-444, 2014-233. University of Chicago IRB 15-0837. Participants gave informed consent to participate in the study before taking part.

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