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Early Increase of Circulating Transitional B Cells and Autoantibodies to Joint-Related Proteins in Patients With Metastatic Melanoma Developing Checkpoint Inhibitor–Induced Inflammatory Arthritis

Mariele Gatto,[1] (iD) Sara Bjursten,[2] Charlotte A. Jonsson,[3] Monica Leu Agelii,[3] (iD) Caroline Jonell,[3] Sarah McGrath,[3] Erik Lönnblom,[4] Outi Sareila,[4] (iD) Rikard Holmdahl,[4] (iD) Anna Rudin,[3] Max Levin,[5] and Inger Gjertsson[3] (iD)

Objective. To investigate potential associations between B cell–related immunologic changes and development of inflammatory arthritis (IA) after treatment with immune checkpoint inhibitors (ICIs).

Methods. Patients who developed ICI-induced IA (ICI-IA) and patients who did not develop immune-related adverse events (non-IRAE) after receiving ICIs to treat metastatic melanoma were consecutively recruited. Blood samples were collected at the time of ICI-IA occurrence and at different time points during treatment. Peripheral blood B cell subsets during ICI treatment were analyzed by flow cytometry. Rheumatoid factor, anti–citrullinated protein antibodies, and antibodies against joint-related proteins were measured.

Results. Proportions of CD19+ B cells were higher in patients with ICI-IA (n = 7) compared to patients with non-IRAE (n = 15) (median 11.7% [interquartile range (IQR) 9.7–16.2%] versus 8.1% [IQR 5.7–11.0%]; $P = 0.03$). The proportion and absolute numbers of transitional CD19+CD10+CD24^{high}CD38^{high} B cells were increased in patients with ICI-IA compared to non-IRAE patients (median 8.1% [IQR 4.9–12.1%] versus 3.6% [IQR 1.9–4.9%]; median 10.7 cells/µl [IQR 8.9–19.6] versus 4.4 cells/µl [IQR 2.3–6.6]; $P < 0.01$ for both). In addition, higher levels of transitional B cells were associated with development of ICI-IA (odds ratio 2.25 [95% confidence interval 1.03–4.9], $P = 0.04$). Transitional B cells increased before the onset of overt ICI-IA and decreased between the active and quiescent stages of ICI-IA ($P = 0.02$). Autoantibodies to type II collagen epitopes were detected in up to 43% of ICI-IA patients compared to none of the non-IRAE patients ($P = 0.02$).

Conclusion. Development of ICI-IA is accompanied by an increase in transitional B cells and by production of autoantibodies to joint-related proteins. Monitoring of B cell–driven abnormalities upon ICI treatment may help earlier recognition of ICI-IA.

INTRODUCTION

Since their approval in 2011, the use of immune checkpoint inhibitors (ICIs) has revolutionized the treatment of several advanced malignancies and especially metastatic melanoma, providing a dramatic prognostic improvement in a substantial proportion of patients (1). ICIs target physiologic immune checkpoint molecules, including programmed death 1 (PD-1), PD ligand 1 (PD-L1), CTLA4, or lymphocyte activation gene 3 protein (LAG3), located on T cells and cognate cells (2–4).

[1]Mariele Gatto, MD, PhD: Department of Rheumatology and Inflammation Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, and Department of Medicine, Unit of Rheumatology, University of Padova, Italy; [2]Sara Bjursten, MD: Department of Oncology, Sahlgrenska University Hospital, and Department of Oncology, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [3]Charlotte A. Jonsson, PhD, Monica Leu Agelii, PhD, Caroline Jonell, MD, Sarah McGrath, PhD, Anna Rudin, MD, PhD, Inger Gjertsson, MD, PhD: Department of Rheumatology and Inflammation Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [4]Erik Lönnblom, PhD, Outi Sareila, PhD, Rikard Holmdahl, MD, PhD: Department of Medical Biochemistry and Biophysics, Section for Medical Inflammation Research, Karolinska Institute, Stockholm, Sweden; [5]Max Levin, MD, PhD: Department of Oncology, Sahlgrenska University Hospital, Gothenburg, Sweden, Department of Oncology, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, and Wallenberg Laboratory for Cardiovascular Research, Department of Molecular and Clinical Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.

Author disclosures and a graphical abstract can be found online at https://onlinelibrary.wiley.com/doi/10.1002/art.42406.

Address correspondence via email to Inger Gjertsson, MD, PhD, at inger.gjertsson@rheuma.gu.se.

Submitted for publication May 30, 2022; accepted in revised form November 8, 2022.

Under physiologic conditions, checkpoint molecules fine-tune the duration and intensity of the adaptive immune response, avoiding uncontrolled immune activation ([2]). Consistently, the removal of checkpoint brakes may result in immune-related adverse events (IRAEs), which comprise a variety of manifestations and may affect any organ system ([5,6]). Rheumatic IRAEs are reported in up to one-third of ICI-treated patients ([5,7,8]). Inflammatory arthritis (IA) occurs in ~5% of all ICI-treated patients with different degrees of severity ([5,6,9]).

Although ICI-induced IA (ICI-IA) may clinically resemble rheumatoid arthritis (RA), the underlying immunologic mechanisms are most likely different ([9–11]). Rheumatoid factor (RF) and anti–citrullinated protein antibodies (ACPAs), hallmarks of RA, are generally absent in ICI-IA ([9,10,12,13]), yet it is not known whether ICI-IA patients develop autoantibodies that recognize other peptides from joint-related proteins. Autoantibodies to such peptides, e.g., type II collagen, present pathogenic as well as regulatory properties in mouse models of arthritis ([14,15]). In addition, they have been shown in patients before the onset of RA in the early stages of disease and have been associated with disease activity ([16–18]). Experimental data have highlighted a likely role for T cells in ICI-IA ([19]), but growing evidence suggests that B cells may also be affected by ICI treatment ([6]). However, so far, no specific data have linked B cells or autoantibody-mediated immunity to ICI-IA.

In this study, we explore changes in the distribution of circulating B cell subsets, presence of autoantibodies against specific joint-protein epitopes, and potential association with development of ICI-IA in patients with metastatic melanoma.

PATIENTS AND METHODS

Patient cohort. We performed a retrospective analysis of prospectively collected data from patients with metastatic melanoma who were treated with ICIs at the Department of Oncology of Sahlgrenska University Hospital (Gothenburg, Sweden) between February 2017 and March 2020. Patients were followed up monthly. Blood samples were obtained throughout the follow-up period according to patient availability and, when possible, a baseline sample was collected immediately before start of ICI treatment; among patients with ICI-IA, blood was also always sampled during active arthritis and at arthritis resolution.

Patients were retrospectively classified as having ICI-IA if they had developed ICI-IA during ICI treatment, or as non-IRAE patients if they had not developed any IRAEs within 6 months after ending ICI treatment. Presence of any preexisting autoimmune rheumatic condition across the patient cohort was assessed through anamnestic data collection, assessment of previous medical records, and clinical examination of the patient.

ICI therapy included anti-CTLA4, anti–PD-1, and anti-LAG3, or any combination thereof according to the oncological treatment schedule (Table [1]). No patients were treated with concomitant ICI therapy and chemotherapy.

ICI-IA. The diagnosis of ICI-IA was confirmed by an experienced rheumatologist at the Rheumatology Department of Gothenburg University. ICI-IA was defined according to clinical judgement based on the clinical evaluation of 66 swollen and 68 tender joints and/or assessed via the 3-variable Disease Activity Score in 28 joints using the C-reactive protein level (DAS28-CRP). The 3-variable DAS28-CRP includes swollen and tender joint count of the 28 evaluated, as well as CRP level, leaving out the patient's assessment of global health ([20]), which renders it suitable for retrospective studies. DAS28-CRP values ≥2.6 were deemed as active arthritis, while DAS28-CRP values <2.6 were deemed as quiescent ([21]). Demographic and clinical parameters including type of treatment, type of IRAE, and time of IRAE onset following ICI initiation were collected from pseudonymized clinical records.

Flow cytometry. Flow cytometry analysis was performed on peripheral blood mononuclear cells (PBMCs). PBMCs were isolated using the Ficoll technique, and nonspecific binding to Fc receptors was prevented by the addition of mouse serum. Staining was performed using the different antibodies listed in Supplementary Table 1 (on the *Arthritis & Rheumatology* website at https://onlinelibrary.wiley.com/doi/10.1002/art.42406).

B cells were defined according to CD19+ expression and categorized into naive (CD27−IgD+), switched memory (CD27+IgD−), non–switched memory (CD27+IgD+), double-negative (CD27−IgD−), and transitional (CD10+CD24^{high}CD38^{high}) B cells. Data were acquired using a BD FACSVerse flow cytometer and analyzed using FlowJo software. An example of gating strategy is given in Supplementary Figure 1 (https://onlinelibrary.wiley.com/doi/10.1002/art.42406).

Detection of circulating autoantibodies. The presence of the autoantibodies ACPAs (determined as antibodies to cyclic citrullinated peptides [anti-CCPs]) and RF was analyzed in clinical routine by the accredited Clinical Immunology Laboratory at Sahlgrenska University Hospital in Gothenburg, Sweden. Anti-CCP was detected using a chemiluminescent microparticle immunoassay (Alinity; Abbott) or using a multiplex bead-based assay (BioPlex 2200 System; Bio-Rad). RF was detected using a fluorometric enzyme-linked immunoassay (Phadia 250; Thermo Fisher Scientific). Positivity was determined according to the manufacturers' instructions. IgG autoantibodies to peptides from joint-related proteins (hereafter referred to as autoantibodies to joint-related proteins) in plasma, including type II collagen, were analyzed using a bead-based flow immunoassay ([22,23]). Briefly, for the 79-plex assay (74 peptides and 5 controls), assayed antigens were coupled to magnetic beads; diluted (1:2) plasma from ICI-IA and non-IRAE patients was added, and bound antibodies

were detected by fluorescently labeled anti-human IgG antibody using a Bio-Plex 200 instrument. Assay results (mean fluorescence intensity [MFI]) for each assayed antigen were compared to each antigen limit of detection (LoD) (24). The sample was deemed positive/detected whenever above the LoD, while samples with an MFI below the LoD were considered negative/not detected and set to a value of zero.

Statistical analysis. Continuous variables were expressed as the mean ± SD or median and interquartile range (IQR), according to their distribution. Mann-Whitney U test was used for comparison between independent samples, while paired samples were compared by Student's *t*-test or Wilcoxon's rank test. Chi-squared test with Fisher's exact test was applied to compare proportion. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated with logistic regression adjusted for confounders. Differences were considered statistically significant when *P* values were less than 0.05. Data were analyzed using GraphPad Prism, version 7, and IBM SPSS Statistics, version 23.

Ethics. This project was conducted according to the Declaration of Helsinki and approved by the Swedish ethical review board (Etikprövningsmyndigheten) (Dnr 151-16, 2016-05-19, T1209-18, 2019-12-01). Patients provided written informed consent for data accessibility for research purposes, and enrollment in this study did not interfere with clinical practice.

RESULTS

Patient cohort. Seven ICI-IA patients and 15 non-IRAE patients were recruited. Demographic and clinical features of patients are reported in Table 1. Lag time from the first (baseline or during treatment) to the subsequent blood sampling suitable for B cell analysis was a mean ± SD of 8.8 ± 4.1 weeks and 8.6 ± 4.8 weeks in the ICI-IA and non-IRAE groups, respectively.

ICI-IA occurred after a median of 14.1 weeks (IQR 2.9–34.0) of ICI initiation. In the majority of cases, ICI-IA was of moderate severity (3-variable DAS28-CRP median value at rheumatology referral 3.50 [IQR 3.02–3.90]) with an RA-like phenotype, most often characterized by symmetric involvement of small joints.

After ICI-IA onset, all patients were treated with glucocorticoids, and 4 of 7 patients also required methotrexate, which was combined with tumor necrosis factor inhibitors in 2 cases. Two patients in the ICI-IA group received an initial combination of anti-CTLA4 and anti–PD-1. One patient received an initial combination of anti-CTLA4 and anti-LAG3 before being switched to anti–PD-1 monotherapy.

Significantly increased circulating B cells in ICI-IA patients. To determine the proportion and absolute numbers of B cell subsets, circulating cells were analyzed by flow cytometry. Data were available for all non-IRAE patients and for 6 of 7 ICI-IA patients.

Table 1. Demographic and clinical features of the ICI-IA and non-IRAE groups*

Characteristic	ICI-IA (n = 7)	Non-IRAE (n = 15)
Female sex, no. (%)	3 (42.9)	7 (46.7)
Age at melanoma diagnosis, mean ± SD years	60.2 ± 14.4	73.8 ± 6.9
ICI regimen (ever), no. (%)		
Anti–PD-1 only	4 (57.1)	15 (100)
Anti-CTLA4 only	0	0
Combination therapy	3 (42.9)	0
Anti–PD-1 plus anti-CTLA4	2	0
Anti–PD-1 plus anti-LAG3	1	0
Lag time from start of ICI treatment to ICI-IA onset, weeks	14.1 (2.9–34.0)	NA
ICI-IA features, no. (%)		
Polyarthritis	6 (85.7)	NA
PMR-like	1 (14.3)	NA
3-variable DAS28-CRP at rheumatology referral	3.50 (3.02–3.90)	NA
TJC (68 joints)	2 (1.25–3.0)	NA
SJC (66 joints)	2 (1.25–2.75)	NA
CRP, mg/liter	53.0 (10.0–95.0)	NA
ACPA and/or RF positivity, no. (%)	0	NA
Treatment for ICI-IA, no. (%)		
Glucocorticoids	7 (100)	NA
Any immunosuppressant medication	4 (57.1)	NA
Methotrexate	4	NA
TNFi	2	NA

* Except where indicated otherwise, continuous data are presented as the median (interquartile range). ICI-IA = immune checkpoint inhibitor–induced inflammatory arthritis; non-IRAE = patients who did not develop immune-related adverse events; PD-1 = programmed cell death protein 1; NA = not applicable; PMR-like = polymyalgia rheumatica–like; DAS28-CRP = Disease Activity Score in 28 joints using the C-reactive protein level (calculated with 3 variables); TJC = tender joint count; SJC = swollen joint count; ACPA = anti–citrullinated antibody; RF = rheumatoid factor; TNFi = tumor necrosis factor inhibitors.

The proportion of circulating B cells was higher in patients with active ICI-IA compared to non-IRAE patients (Table 2). The proportion of circulating CD19+ B cells in patients receiving stable anti–PD-1 monotherapy or having received combination therapy prior to monotherapy did not differ significantly (median 14.7% [IQR 8.2–20.5] versus 11.7% [IQR 10.2–11.8%]; P = 0.7). In non-IRAE patients, circulating CD19+ B cells decreased from treatment initiation to week 8 of treatment (median 7.9% [IQR 5.7–12.4%] versus 6.4% [IQR 4.3–10.7%]; P = 0.002), while there was no significant change in circulating B CD19+ B cells among ICI-IA patients (median 10.0% [IQR 6.7–12.3%] versus 11.9% (10.2–14.0%]; P = 0.25).

Increase of transitional B cells in ICI-IA patients. The proportion and absolute numbers of circulating transitional B cells (CD10+CD24^{high}CD38^{high}) were expanded in patients with active ICI-IA compared to those with non-IRAE (Figures 1A and B and Table 2). The exclusive enrichment in the proportion of transitional B cells was associated with development of ICI-IA (OR 2.2 [95% CI 1.0–4.9], P = 0.04) (Figure 1C). There were no observed differences between patients with ICI-IA and those with non-IRAE with respect to the populations of naive (CD27–IgD+), memory (CD27+IgD+/–), and double-negative (CD27–IgD–) B cells (Table 2).

Increase in circulating transitional B cells before ICI-IA onset and consistency with ICI-IA activity. The increase of circulating transitional B cells in patients with ICI-IA who had available baseline samples was apparent after a median 8.0 weeks (IQR 6.1–11.1) from treatment start. The clinical onset of ICI-IA was documented after a median 14.4 weeks (IQR 7.9–24.1) in those patients and a median 14.1 weeks (IQR 2.9–34.0) weeks across the whole ICI-IA group (Table 1). Proportions of transitional B cells in ICI-IA patients decreased between the active and the quiescent phase of ICI-IA (median 7.2% [IQR 4.7–12.1%] versus 3.8% [IQR 1.6–7.6%]; P = 0.02), whereas the proportion of total B cells did not change (median 11.7% [IQR 9.2–16.2%] versus 12.0% [IQR 10.9–13.9%]; P = 0.97) (Figure 2).

Age-related changes across the B cell pool. Patients with ICI-IA were significantly younger at the time of melanoma diagnosis compared to non-IRAE patients (P = 0.007), and we thus investigated possible correlations between age and B cell changes, as reported in Supplementary Table 2 (https://onlinelibrary.wiley.com/doi/10.1002/art.42406). Whereas the overall B cell pool and major mature populations do not exhibit significant correlations, the proportion of circulating transitional cells shows a borderline moderate inverse correlation with age (r = –0.48, P = 0.03).

Differential expression of autoantibodies to joint-related proteins between ICI-IA patients and non-IRAE patients. We analyzed whether IgG autoantibodies to native or citrullinated joint-related proteins were detected in plasma from ICI-IA and non-IRAE patients. Of 74 different joint-related epitopes, autoantibodies to 36 (48%) of these were detected. A significantly increased proportion (43% versus 0%; P = 0.023) of ICI-IA patients displayed reactivity against the cyclic citrullinated epitope 19 in joint protein 5 (JP5-C-19-CIT), and a trend was detected toward an increased proportion (28% versus 0%;

Table 2. Circulating B cell proportions and cell counts in patients with active ICI-IA and those with non-IRAE*

	ICI-IA (n = 6)	Non-IRAE (n = 15)	P
CD19+ B cells			
Median (IQR) % (on total)	11.7 (9.7–16.2)	8.1 (5.7–11.0)	0.03†
Median (IQR) cells/μl	154.5 (90.0–222.0)	94 (85–154.5)	0.24
CD27+IgD–	5.3 (3.7–8.4)	8.2 (4.5–14.4)	0.96
Median (IQR) % (on CD19+)	7.6 (5.5–14.2)	8.1 (5.0–14.5)	0.88
Median (IQR) cells/μl			
CD27+IgD+	2.1 (1.1–16.8)	5.6 (2.1–7.8)	0.99
Median (IQR) % (on CD19+)	4.0 (1.5–21.8)	5.2 (2.7–11.7)	0.89
Median (IQR) cells/μl			
CD27–IgD+	57.2 (49.4–66.2)	55.8 (50.2–62.4)	0.97
Median (IQR) % (on CD19+)	84.7 (50.6–140.7)	60.4 (51.9–125.7)	0.90
Median (IQR) cells/μl			
CD27–IgD–	2.7 (2.3–3.8)	4.9 (3.6–8.4)	0.67
Median (IQR) % (on CD19+)	3.8 (2.4–7.5)	5.1 (2.9–13.8)	0.78
Median (IQR) cells/μl			
CD24–CD38+	0.29 (0.1–0.6)	0.18 (0.07–0.3)	0.99
Median (IQR) % (on CD19+)	0.28 (0.1–0.6)	0.18 (0.08–0.3)	0.99
Median (IQR) cells/μl			
CD10+CD24^{high}CD38^{high}	8.1 (4.9–12.1)	3.6 (1.9–4.9)	0.007†
Median (IQR) % (on CD19+)	10.7 (8.9–19.6)	4.4 (2.3–6.6)	0.0013†
Median (IQR) cells/μl			

* IQR = interquartile range (see Table 1 for other definitions).
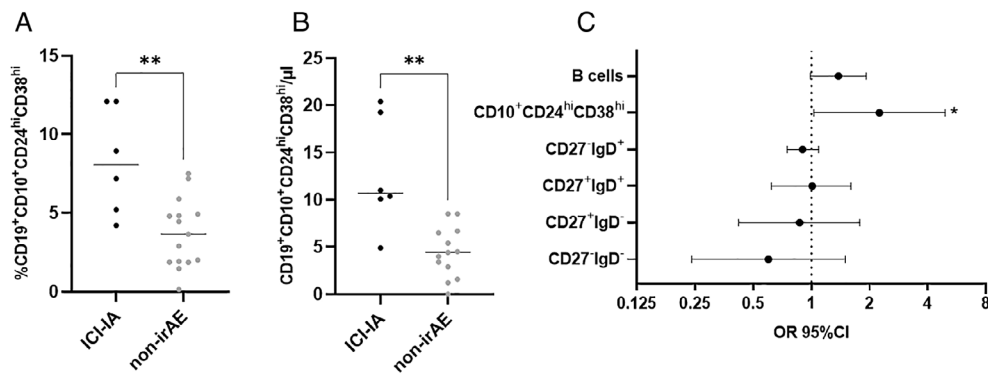† Between-group differences were significant at P < 0.05.

Figure 1. Transitional B cells increased in patients with immune checkpoint inhibitor–induced inflammatory arthritis (ICI-IA). **A** and **B,** The proportion (**A**) and absolute numbers (**B**) of circulating transitional B cells (CD19+CD10+CD24^{high}CD38^{high}) in active ICI-IA compared to patients who did not develop immune-related adverse events (non-IRAE). Bars show the median. **C,** Forest plot showing B cell changes in relation to the onset of ICI-IA. ** = $P < 0.01$ by Mann-Whitney U test (**A** and **B**) or by multivariable logistic regression adjusted for reciprocal changes relative to the B cell pool (**C**). OR = odds ratio; 95% CI = 95% confidence interval.

$P = 0.09$) of ICI-IA patients positive for autoantibodies against the cyclic citrullinated type II collagen epitope C1 (CII-C-C1-CIT-R) (Figure 3A). Although not reaching statistical significance, a lower proportion of ICI-IA patients compared to non-IRAE patients showed reactivity against the triple helical arginine epitope F4 derived from type II collagen (CII-T-F4-R-R) (28% versus 47%; $P = 0.65$) (Figure 3A). The MFI levels of these autoantibodies, which were detected in the ICI-IA patients, were well above the LoD, indicating true reactivity. The response to CII-T-F4-R-R was reduced in ICI-IA patients, while the intensity of the reactivities to

JP5-C-19-CIT and CII-C-C1-CIT-R were increased compared to non-IRAE patients (Figure 3B).

DISCUSSION

In this study, we explored the circulating B cell pool and have identified potentially protective or pathogenic autoantibodies in patients with metastatic melanoma treated with ICIs, with a focus on patients developing ICI-IA. In fact, despite the fact that ICI-IA significantly jeopardizes the quality of life of patients already burdened with a metastatic malignancy, its recognition is often overlooked (12); therefore, the discovery of handy biomarkers to monitor in patients receiving ICI therapy is of utmost importance. In this regard, we show that patients with ICI-IA, but not those belonging to the non-IRAE group, display a significant increase in the transitional B cell subset, which eventually decreases between the active and the quiescent phase of ICI-IA. Furthermore, we document that patients who develop ICI-IA have increased levels of potentially pathogenic antibodies and decreased levels of potentially protective antibodies to joint-related proteins (23,25) compared to non-IRAE patients.

Normal levels of circulating transitional cells in healthy adult subjects make up 2–3% of the total B cells (26). In our cohort, patients with active ICI-IA displayed up to 12% of circulating transitional B cells compared to a median of 3.6% among non-IRAE patients, which suggests that their persistent elevation may be associated with an overt autoimmune reaction. Importantly, the increase in transitional B cells preceded clinical onset and was independently associated with the development of ICI-IA. It can be argued that patients classified as non-IRAE could develop IRAEs in the future. However, in the present study, we investigated the early B cell–related abnormalities during ICI treatment that could accompany ICI-IA, thus making it reasonable to compare patients with overt ICI-IA to those who were IRAE-free
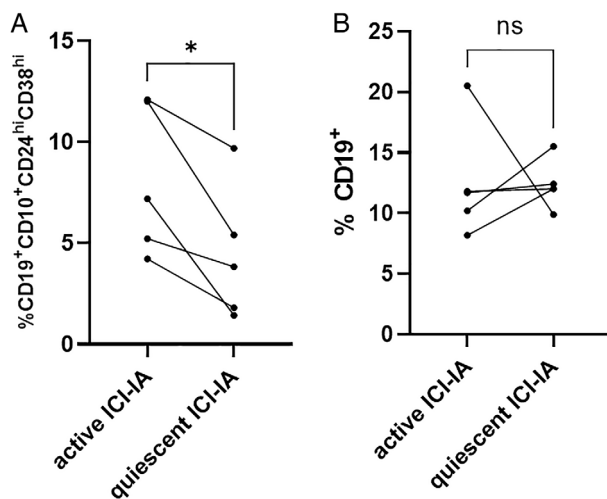

Figure 2. Circulating transitional B cells increase prior to immune checkpoint inhibitor–induced inflammatory arthritis (ICI-IA) onset. The proportion of circulating transitional B cells (**A**) and total B cells (**B**) in patients with active and quiescent ICI-IA. Active ICI-IA is defined as having a 3-variable Disease Activity Score in 28 joints using the C-reactive protein level (DAS28-CRP) of ≥2.6 and quiescent ICI-IA as a DAS28-CRP score of <2.6. * = $P < 0.05$ by t-test for paired samples. NS = not significant.
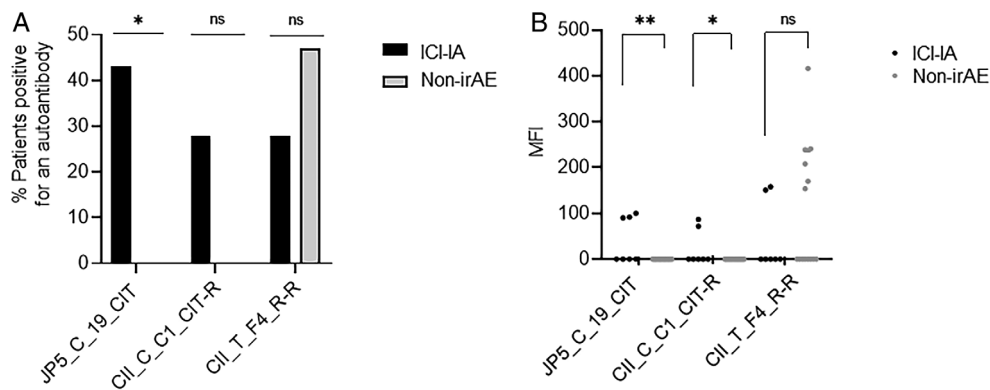
Figure 3. Autoantibodies to joint-related proteins were differentially expressed between ICI-IA patients and non-IRAE patients. The proportion of ICI-IA patients and non-IRAE patients having sera reactivity for the indicated peptides (**A**) and the corresponding MFI levels (**B**). In **B**, whenever the mean fluorescence intensity (MFI) was below the limit of detection, it was given a value of 0 to visualize and calculate the statistics. * = $P < 0.05$; ** = $P < 0.01$, by independent *t*-test and Fisher's exact test. JP5-C-19-CIT = cyclic citrullinated epitope 19 in joint protein 5; CII-C-C1-CIT-R = cyclic citrullinated type II collagen epitope C1; CII-T-F4-R-R = triple helical arginine epitope F4 derived from type II collagen; NS = not significant (see Figure 1 for other definitions).

at the time of comparison and 6 months after stopping the treatment.

Transitional B cells are released from the bone marrow and represent an intermediate stage of maturation before progression to the mature splenic or follicular B cell pool (27,28). Under physiologic conditions, transitional cells are subjected to mechanisms of peripheral selection that reduce the chances for autoreactive B cells to escape central selection (27,29). However, pitfalls at the transitional checkpoint have been described in several autoimmune rheumatic diseases (30,31), in which increased numbers of transitional B cells are rescued into the mature B cell pool. Therefore, it may be speculated that an expansion of transitional B cells reflects a potential enlargement in the autoreactive B cell pool, whose maturation might be aided by the loosening of checkpoint control, thereby leading to overt autoimmunity.

Demographic features in our cohort are consistent with previous results in terms of age and sex distribution (32,33). The patients who developed ICI-IA were significantly younger than those who did not, which is consistent with previously published findings that indicate that the incidence of IRAEs is higher in younger patients (34,35). Additionally, it is worth noting that the proportion of circulating transitional B cells displayed a moderately negative correlation with age, consistent with reports on age-related B cell kinetics (36), suggesting that younger patients might be more prone to ICI-related side effects on an immunologic basis. However, the sample size in our study as well as the presence of potential outliers might hamper the accuracy of this statistical relationship, as suggested by the great range of confidence intervals. In fact, to which extent age accounts for ICI-related changes within the B cell pool and for the occurrence of joint-specific antibodies seen in our patients remains to be defined in a larger cohort. While the search for independent clinical predictors of ICI-IA goes beyond the scope of our study, this

observation deserves further investigation of physiologic age-related changes in the adaptive immune pool as well as of any age-driven modification in the expression of certain checkpoint molecules on T and B cells (37), which might interject ICI-related effects.

Consistent with previous reports (9,38,39), ICI-IA patients in our cohort were negative for RF as well as for anti-CCP antibodies, which make up the commonly measured citrullinated epitopes for ACPA assays in clinical practice. Herein, we demonstrate for the first time that circulating IgG autoantibodies, which recognize other citrullinated joint-related proteins (40,41), are present in ICI-IA patients. Autoantibodies against the citrullinated epitopes CII-C-C1-R-CIT and JP5-C-19-CIT were significantly increased, and autoantibodies to CII-T-F4-R-R were decreased in ICI-IA patients compared to non-IRAE patients. Interestingly, these autoantibodies have been described in a proportion of patients with early RA (18), and autoantibodies to CII-C1 could induce arthritis (40), while anti-CII F4 autoantibodies dampened arthritis development in mouse models of RA (14), thereby potentially playing a role in ICI-IA establishment or prevention. These results could indicate either an increased general B cell activation or a specific regulated immune response to joint-related proteins as a part of ICI-IA disease mechanisms.

It should be noted that 3 of 7 ICI-IA patients underwent a short course of combination treatment before being switched to anti–PD-1 monotherapy. Even though combination treatment more frequently triggers IRAEs (42), no difference in circulating B cells was documented during the active phase of ICI-IA between combination therapy and monotherapy, suggesting that B cell changes might be a marker for ICI-IA regardless of the underlying treatment.

A role for B cells, beside T cells, in ICI-related autoimmunity has recently been suggested by data linking circulating B cells to

different types of IRAEs (43) and by the occurrence of organ-specific antibodies in patients developing IRAEs (44). Moreover, targeting B cells with the anti-CD20 antibody rituximab reduced the frequency of ICI-related hypothyroidism (44,45), and clinical trials that combine B cell depletion with ICI treatment to prevent IRAEs are ongoing (46), giving credence to the notion of B cell involvement in ICI-IRAE.

Limitations of this study include the small sample size and the lack of examination of the inflamed synovium and of functional assays aimed at investigating potential regulatory properties within the transitional B cell pool. Additionally, no data are available about any preexisting positive autoantibodies in sera of patients who developed ICI-IA, as their measurement is not routinely recommended before ICI therapy (47). On the other hand, this is the first study that investigates abnormalities in the circulating B cell compartment and autoantibody response in relationship to ICI-IA, suggesting potential B cell–sustained mechanisms of ICI-IA and submitting changes in specific B cell subsets as possible biomarkers for IRAE susceptibility.

In summary, our findings demonstrate that development of ICI-IA is associated with an early expansion of transitional CD10+CD24^{high}CD38^{high} B cells and is accompanied by production of autoantibodies to joint-related proteins in a proportion of patients. Monitoring of circulating transitional CD10+CD24^{high}CD38^{high} B cells and detection of specific joint-directed antibodies upon ICI initiation may facilitate identification of patients at risk of ICI-IA, for whom closer monitoring is warranted.

ACKNOWLEDGMENT

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Gjertsson had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study conception and design. Levin, Gjertsson.
Acquisition of data. Gatto, Bjursten, Jonsson, Jonnell, Lönnblom, Sareila, Levin, Gjertsson.
Analysis and interpretation of data. Gatto, Agelii, McGrath, Sareila, Holmdahl, Rudin, Levin, Gjertsson.

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