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Autoreactive B cells remain active despite clinical disease control in rheumatoid arthritis

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ABSTRACT

Background: Autoimmune diseases (AIDs) are frequently hallmarked by the presence of autoreactive B cell responses which are involved in disease pathogenesis. However, the dynamics of such responses and their relation to clinical disease activity in humans is poorly understood. Rheumatoid arthritis (RA), a prototypic chronic AID, is hallmarked by B cell responses directed against citrullinated proteins.
Objective: To determine the relation between the activity of the anti-citrullinated protein antibody (ACPA) B cell response and clinical disease activity in ACPA$^+$ patients with RA.
Methods: Expression of B cell activation markers by ACPA$^+$, tetanus toxoid (TT)$^+$ and ACPA$^-$ memory B cells (MBCs) from peripheral blood of ACPA$^+$ RA patients receiving different treatments was analyzed by flow cytometry. Results were correlated to clinical disease activity.
Results: Compared to TT$^+$ and ACPA$^-$ MBCs, ACPA$^+$ MBCs displayed a highly activated phenotype as evidenced by increased expression of Ki-67, CD86, CD80, CD19 and CD20 and reduced expression of CD32. The activated phenotype of ACPA$^+$ MBCs did not associate with clinical disease activity in a cross-sectional analysis of RA patients treated with various therapeutic agents. Also, in a longitudinal analysis of patients treated with Janus kinase (JAK) inhibitors, ACPA$^+$ MBCs retained their activated phenotype despite effective control of inflammation and clinical disease.
Conclusion: ACPA$^+$ MBCs remain active despite clinical disease control in patients with RA across a range of interventions. This persistent activity indicates the absence of immunological remission and might explain why ACPA$^+$ patients rarely reach sustained drug-free remission and frequently flare upon drug tapering.

1. Introduction

Chronic autoimmune diseases (AIDs) can be managed increasingly well with medications that effectively inhibit inflammation [1]. However, these therapeutics are non-curative and often require life-long administration to prevent disease flares. Patients rarely reach sustained drug-free remission, currently considered the closest proxy for cure. This indicates that current treatment modalities do not effectively silence the immune responses and/or tissue-specific processes that cause inflammation but chronically persist even when disease appears clinically in remission under treatment [2,3]. The underlying mechanisms for the persistence of autoimmune responses are poorly understood, and markers reflecting the activity of disease-specific immunological processes are lacking.

Many AIDs are hallmarked by autoreactive B cell responses and are responsive to therapeutic B cell targeting [4,5]. So far, most insights into autoreactive B cell responses are based on the analysis of circulating autoantibodies in the disease context [6]. However, the potential of autoantibodies to accurately reflect the activity and dynamics of the underlying B cell responses is limited due to the relatively long half-life of antibodies and the variety of cells secreting antibodies ranging from recently activated plasmablasts to long-lived, bone marrow-resident plasma cells [7,8]. In contrast, cellular analyses of antigen-specific B cells can trace the activation of B cell responses directly [9,10]. For example, exposure to microbial antigens following vaccination or infection is marked by the appearance of activated memory B cells (MBCs) and plasmablasts in the circulation within days and a subsequent phase of restoration of immunological 'quiescence' within weeks or

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months [9,10]. In the case of AIDs, analyses of antigen-specific B cells can help to understand the persistence and functional dynamics of autoreactive B cell memory which may underly chronicity of disease.

Rheumatoid arthritis (RA) is a chronic AID characterized by the prototypical features described above. 50–70% of patients harbor autoreactive B cells targeting citrullinated protein antigens. Anti-citrullinated protein antibodies (ACPA) have emerged as important prognostic and diagnostic biomarkers. The presence and evolution of the ACPA B cell response is closely linked to disease onset, the risk for disease flares and a low incidence of drug-free sustained remission [11–14]. B cell depletion is highly effective in RA, especially in the ACPA$^+$ patient subgroup [15,16]. Previously, our group could show that patients with chronic RA harbor ACPA$^+$ B cells that display a proliferative and activated phenotype [17]. This phenotype contrasted a less active state in the phase preceding disease. Together, these data suggest that the ACPA B cell response in RA might reflect immunological disease activity more closely than the secreted ACPA repertoire, and that it might by itself be a marker and even driver of persistent immunological disease.

Here, we studied the dynamics of the ACPA B cell response regarding its capacity to reflect immunological disease activity. Specifically, we analyzed whether the degree of activation of the ACPA B cell response varies with clinical measures of disease activity in patients receiving different disease-modifying anti-rheumatic drugs (DMARDs). We found a remarkable activity of antigen-specific B cell autoimmunity independent of clinical disease activity despite anti-inflammatory treatment, and thereby provide a rationale to target these cells and/or their triggers specifically to achieve sustained remission without disease flares.

2. Methods

2.1. Patient inclusion

Peripheral blood was obtained from ACPA$^+$ RA patients (n = 49) recruited from the outpatient clinic of the department of Rheumatology at Leiden University Medical Center. All patients met the ACR/EULAR 2010 classification criteria for RA at the time of diagnosis and were ACPA-IgG positive. All samples were collected and worked-up directly ex vivo without freezing cells in between.

For the cross-sectional analysis, a variety of RA patients were included from the outpatient clinic of the department of Rheumatology at Leiden University Medical Center (Table 1). Patients were either on classical synthetic DMARDs (csDMARDs) (methotrexate monotherapy or in combination with sulfasalazine or hydroxychloroquine) or were stably treated with tumor necrosis factor-α (TNF-α) inhibitors (monotherapy or in combination with methotrexate, sulfasalazine or hydroxychloroquine) for at least two months (median: 42 months, range: 2–201 months).

For the longitudinal analysis, patients starting on Janus kinase (JAK) inhibitors were included based on the decision of the patient's treating rheumatologist to start a JAK inhibitor for medical reasons (active disease), either as monotherapy or as add-on to existing csDMARD medication (Table 1).

Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids were allowed in both settings. Clinical disease activity was determined at the day of blood withdrawal by a trained rheumatologist by determining the swollen and tender joint counts. Erythrocyte sedimentation rate (ESR) was measured as part of routine clinical care. A composite disease activity score (DAS28(3v)) was calculated based on these three variables. If criteria for inclusion in the cross-sectional analysis were met, baseline measurements for the longitudinal analysis were also included in the cross-sectional analysis. As to our knowledge, none of the individuals received a recent tetanus vaccination prior to recruitment.

2.2. Fluorescent labeling of antigens

The antigens cyclic citrullinated peptide 2 (CCP2) and tetanus toxoid (TT) were fluorescently labeled as described previously [17]. In short, CCP2-biotin was tetramerized by streptavidin-APC (InVitrogen) and -BV605 (Biolegend), while cArgP2-biotin was linked to ExtrAvidin-PE (Sigma-Aldrich). TT (Statens Serum Institut) was coupled to APC and PE using Anatag™ APC and PE labeling kits (AnaSpec) in accordance with the manufacturer's instructions. CCP2 and cArgP2 tetramers were titrated for optimal concentrations on ACPA-expressing human embryonic kidney cells stably transfected with a surface-expressed ACPA-IgG (HEK^{TM}) [18]. Fluorescently labeled TT was titrated on an immortalized TT-reactive B cell line.

Table 1
Patient characteristics.

	Patients included in cross-sectional analysis (n = 42)	Baseline characteristics of patients starting on JAK inhibitors (n = 13)
Age (years)[a]	64 (55–69)	67 (61–69)
Sex, female subjects, n	33	11
Disease duration, years[a]	11 (3–17)	13 (6–23)
DAS28(3v)[a]	2.87 (1.86–4.40)	5.00 (3.95–5.73)
28 swollen joint count[a]	1 (0–5)	8 (4–9)
28 tender joint count[a]	0.5 (0–4)	7 (6–8)
Erythrocyte sedimentation rate (mm/hr)[a]	14 (6–31)	31 (14–43)
ACPA-IgG (aU/ml)[a]	1828 (908–2720)	1453 (440–2015)
Rheumatoid factor +, n	40	12
csDMARDs, n	36	11
• Methotrexate, n	32	6
• Hydroxychloroquine, n	9	2
• Sulfasalazine, n	10	2
• Leflunomide, n	0	3
TNF-α inhibitors, n	22	5
• Etanercept, n	12	4
• Adalimumab, n	8	1
• Golimumab, n	2	0
Sarilumab, n	0	1
Abatacept, n	0	1
Prednisone, n	6	4
JAK inhibitor initiated at baseline	N/A	13
• Tofacitinib, n		6
• Baricitinib, n		5
• Filgotinib, n		2
DMARD use concomitant with JAK inhibitor, n	N/A	10
• Methotrexate, n		4
• Hydroxychloroquine, n		2
• Sulfasalazine, n		2
• Leflunomide, n		3
Prednisone use concomitant with JAK inhibition, n	N/A	6

[a] Values represent median (interquartile range).

2.3. Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll-Paque gradient centrifugation. PBMCs were separated in fractions to stain for ACPA$^+$ B cells (45% of PBMCs), TT$^+$ B cells (35% of PBMCs) and to perform fluorescence minus one (FMO) controls (20% of PBMCs). Upon staining with Live/Dead™ Fixable Aqua Stain Kit (InVitrogen), PBMCs were stained with antibody mixes containing anti-CD3-BV510 (UCHT1, BioLegend), anti-CD14-BV510 (M5E2, BioLegend), anti-CD19-APC-Cy7 (SJ25C1, BD Biosciences), anti-CD20-FITC (2H7, BioLegend), anti-CD27-BV421 (M-T271, BD Biosciences), anti-CD32-BV786 (FLI8.26, BD Biosciences), anti-CD80-APC-R700 (L307.4, BD Biosciences), anti-CD86-PE-Cy7 (FUN1, BD Biosciences) together with the fluorescently labeled antigens CCP2-APC, CCP2-BV605, CArgP2-PE or TT-APC and TT-PE. Cells were subsequently fixed, permeabilized (Foxp3/Transcription Factor Staining Buffer Set, Invitrogen™) and stained with anti-Ki-67 PerCP-eFluor710 (20Raj1, eBioscience).

FMO stainings were performed using the panel to identify ACPA$^+$ B

cells, but without the addition of anti-CD32, anti-CD80, anti-CD86 or anti-Ki-67. Cells were processed on a BD LSRFortessa X-20 4L flow cytometer (BD Biosciences) which was set-up according to EuroFlow guidelines (www.EuroFlow.org). Flow cytometer was calibrated daily with BD Biosciences™ Cytometer Setup and Tracking beads (BD Biosciences) and flow cytometer performance was verified before acquisition with SPHERO™ Rainbow Calibration Particles (Spherotech). Data were analyzed using BD FACSDiva™ (version 9.0) and FlowJo software (version 10.9.0).

A cut-off was applied to determine the minimum number of ACPA$^+$ MBCs required for phenotypical analysis. Patients were included in the cross-sectional analysis if the ACPA$^+$ MBC frequency as percentage of total B cells exceeded 6,9x10^{-4} (the mean frequency of MBCs reactive towards the tetramerized CCP2 antigen measured in seven ACPA$^-$ control patients, plus two times the standard deviation). Additionally, a numerical cut-off was added limiting the analysis to the presence of at least seven ACPA$^+$ MBCs to prevent skewing of the data due to low cell numbers. This numerical cut-off was determined based on repeated analyses of data with high and low cell numbers in which stability of data was lost if less than 7 cells were available for analysis. Patients starting on JAK inhibitors were included if the cut-off was reached at baseline and if there was at least one follow-up measurement. As for ACPA, TT$^+$ MBCs were phenotypically analyzed if the TT$^+$ MBC frequency was higher than 6.9x10^{-4} and if there were more than seven TT$^+$ MBCs.

2.4. ACPA-IgG ELISA

Levels of circulating ACPA-IgG were determined by ELISA based on the reactivity of plasma IgG towards CCP2. High Bind 384-well Microplates (Corning) were coated with 1 μg/ml streptavidin (InVitrogen) and 1 μg/ml CCP2-biotin or CArgP2-biotin (Leiden University Medical Center) was added subsequently. Heparin plasma was applied in a dilution of 1:50. Saturated samples were diluted further. IgG was detected using 1:5000 diluted goat anti-human IgG-HRP (DAKO, P0214) with ABTS as substrate. Pooled serum samples of ACPA$^+$ RA patients served as standard.

2.5. Statistical analysis

Cross-sectional data were analyzed using Mann-Whitney U or Kruskal-Wallis tests combined with Dunn's multiple comparisons test, as indicated in the figure legends. Correlations were described using the nonparametric Spearman's rank correlation coefficient. The evolution of continuous outcomes in the longitudinal analysis of patients treated with JAK inhibitors was assessed with linear mixed effects models. Patient identifier was considered a random effect, while time was the only fixed effect. Models with a random intercept and a fixed slope were compared by likelihood ratio test with models with a random intercept and a random slope. In case of non-inferiority, the model with a random intercept and a fixed slope was chosen. Outcomes expressed as percentage were analyzed with beta regression with patient identifier as a random and time as a fixed effect. In order to evaluate the influence of time on outcomes, all models were compared to null-models that did not contain time as a fixed effect. Statistical analyses on the cross-sectional data were performed using Graphpad Prism (version 9.3.1.). Models were generated with the packages nlme and GLMMadaptive in R (version 4.2.1).

2.6. Study approval

The study was approved by the ethical review board of Leiden University Medical Center under protocol number P17.151. All patients gave written informed consent for study participation.

3. Results

3.1. ACPA$^+$ MBCs display a highly activated and proliferative phenotype in RA patients

As an initial step, we cross-sectionally phenotyped ACPA$^+$ B cells in the circulation of ACPA$^+$ RA patients (Table 1). Patients on csDMARDs and/or TNF-α inhibitors (either as monotherapy or combined with csDMARDs) were included to obtain a representative group of patients encountered in daily clinical practice. ACPA$^+$ B cells were identified using fluorescently labeled CCP2-streptavidin tetramers (Supplementary Fig. 1A), as described before [17]. The phenotype of these cells was compared to TT$^+$ B cells which are representative of a protective, resting, recall MBC response, and to ACPA$^-$ B cells which did not bind to tetramerized CCP2 (Supplementary Fig. 1A).

The frequency of ACPA$^+$ and TT$^+$ B cells ranged between 1:1.000 and 1:10.000 of the total B cell population (Supplementary Figs. 2A–B). In line with previous results [17], the majority of ACPA$^+$ and TT$^+$ B cells displayed a MBC phenotype as they co-expressed CD20 and CD27 (Supplementary Fig. 1B, Supplementary Figs. 2C–E). Few ACPA$^+$ B cells displayed characteristics of naive B cells (CD20$^+$CD27$^-$) or plasmablasts (CD20^{low}CD27^{++}). Frequencies of ACPA$^+$ B cells and ACPA$^+$ MBCs correlated with levels of circulating ACPA-IgG (Supplementary Figs. 2F–G).

To limit variation due to the heterogeneity of B cell subsets, we subsequently focused on ACPA$^+$ MBCs. This predominant subset may be most relevant for the persistence of immunological memory and, possibly, disease chronicity. Various activation markers were used to phenotype antigen-specific MBCs: proliferation marker Ki-67, T cell co-stimulatory molecules CD80 and CD86, regulatory Fc-gamma receptor CD32; and prototypical B cell markers CD19 and CD20 (Supplementary Fig. 1C). Based on expression levels of these markers, ACPA$^+$ MBCs displayed a highly activated and proliferative phenotype in RA patients in the cross-sectional analysis (Fig. 1). Expression levels of Ki-67, CD86,

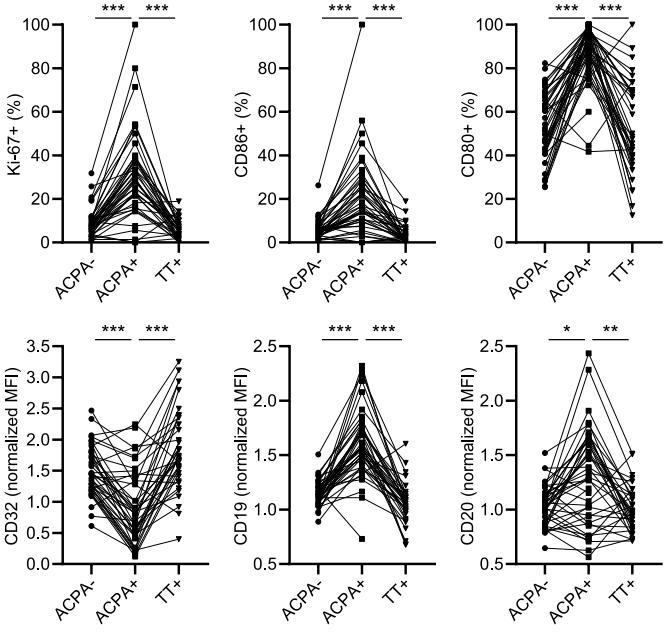

Fig. 1. Expression of activation markers by ACPA$^-$, ACPA$^+$ and TT$^+$ MBCs. Expression of Ki-67, CD86, CD80, CD32, CD19 and CD20 by ACPA$^-$, ACPA$^+$ and TT$^+$ MBCs in a cross-sectional analysis of RA patients. Median fluorescence intensities (MFIs) were normalized to MFIs expressed on non-antigen-specific (ACPA$^-$/TT$^-$) CD20$^+$CD27$^-$ B cells (naive). Kruskal—Wallis test with Dunn's multiple comparisons test was used to test whether ACPA$^+$ MBCs statistically differed from ACPA$^-$ or TT$^+$ MBCs. ns = non-significant, * = $p < 0.05$, ** = $p < 0.005$, *** = $p < 0.001$.

CD80, CD19 and CD20 were higher, while CD32 expression was lower on ACPA$^+$ MBCs compared to TT$^+$ and ACPA$^-$ MBCs.

3.2. The activated phenotype of ACPA$^+$ MBCs is not linked to clinical disease activity in RA patients treated with various therapeutic agents

Variation was observed in the expression levels of individual activation markers on ACPA$^+$ MBCs between patients. We hypothesized that this could relate to clinical disease activity, as the activation of antigen-specific MBCs has been associated with disease severity in the context of infectious diseases like severe-acute-respiratory-syndrome-related coronavirus 2 (SARS-CoV-2) disease, human immunodeficiency virus (HIV)-mediated acquired immunodeficiency syndrome and hepatitis B [19–23]. Both frequency of ACPA$^+$ MBCs and levels of circulating ACPA-IgG did not correlate with clinical disease activity (Fig. 2A and B). Also, no significant correlations were observed between activation markers expressed by ACPA$^+$ MBCs and clinical disease activity (Fig. 2C). Furthermore, stratification of patients based on treatment with either csDMARDs only or TNF-α inhibitors (as monotherapy or combined with csDMARDs) did not show significant correlations (Fig. 3A and B). We also assessed the relation between the expression of activation markers by ACPA$^+$ MBCs and ACPA-IgG levels in plasma, as the frequency of ACPA$^+$ MBCs correlates with these levels. The activation markers expressed by ACPA$^+$ MBCs did not (or only weakly) correlate with ACPA-IgG levels and strong interindividual variations were observed (Supplementary Fig. 3). Although weak correlations were significant for CD80 and CD32 (Spearman's rank correlation coefficients of 0.36 and −0.34, respectively), patients with lower ACPA-IgG levels still displayed highly activated ACPA$^+$ MBCs. Together, these findings indicate that the ACPA B cell response and its activation resist clinical disease control with anti-inflammatory agents widely used in clinical practice.

3.3. JAK inhibitors do not revert the activation of autoreactive B cells towards a resting state despite effective control of clinical disease

The persistence of the ACPA B cell response and its activity indicate continuous exposure of ACPA$^+$ B cells to stimulating factors that cannot be controlled by csDMARDs or TNF-α inhibitors. JAK inhibitors have emerged as effective DMARDs which inhibit intracellular signaling of many pro-inflammatory cytokines, like IL-6, IL-21, and interferons [24]. As these cytokines are involved in the activation of B cell responses, we next assessed whether the phenotype of ACPA$^+$ B cells would be affected upon administration of JAK inhibitors. To this end, we analyzed the phenotype of ACPA$^+$ B cells from 13 RA patients before and at two timepoints during JAK inhibitor treatment with a maximum follow-up of 210 days (Table 1). For two patients, only one follow-up measurement was available due to premature discontinuation/inefficacy of JAK inhibition (n = 1) or loss to follow-up (n = 1). Initiation of JAK inhibitor treatment led to a clear decrease in clinical disease activity (Fig. 4A). Nonetheless, the frequencies of ACPA$^+$ and TT$^+$ MBCs and levels of ACPA-IgG in plasma remained unchanged. (Fig. 4B–D). The expression

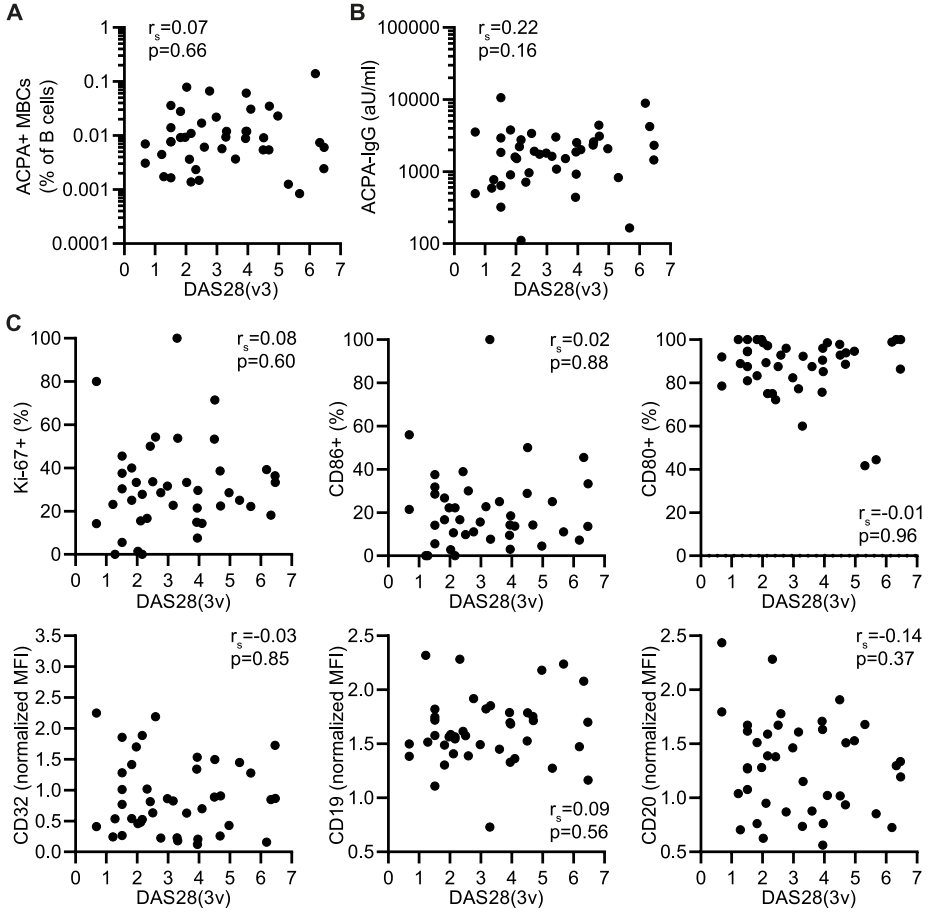

Fig. 2. Correlation between the expression of activation markers by ACPA$^+$ MBCs and clinical disease activity.
(A–C) Correlation between the frequency of ACPA$^+$ MBCs as a percentage of total B cells and clinical disease activity (DAS28(3v)) (A), circulating ACPA-IgG expressed in arbitrary units per ml (aU/ml) and DAS28(3v) (B) and expression of Ki-67, CD86, CD80, CD32, CD19 and CD20 by ACPA$^+$ MBCs and DAS28(3v) (C) in a cross-sectional analysis of RA patients. Median fluorescence intensities (MFIs) were normalized to MFIs expressed on non-antigen-specific (ACPA$^-$/TT$^-$) CD20$^+$CD27$^-$ B cells (naive). Correlations were described using Spearman's rank correlation coefficient (r_s).

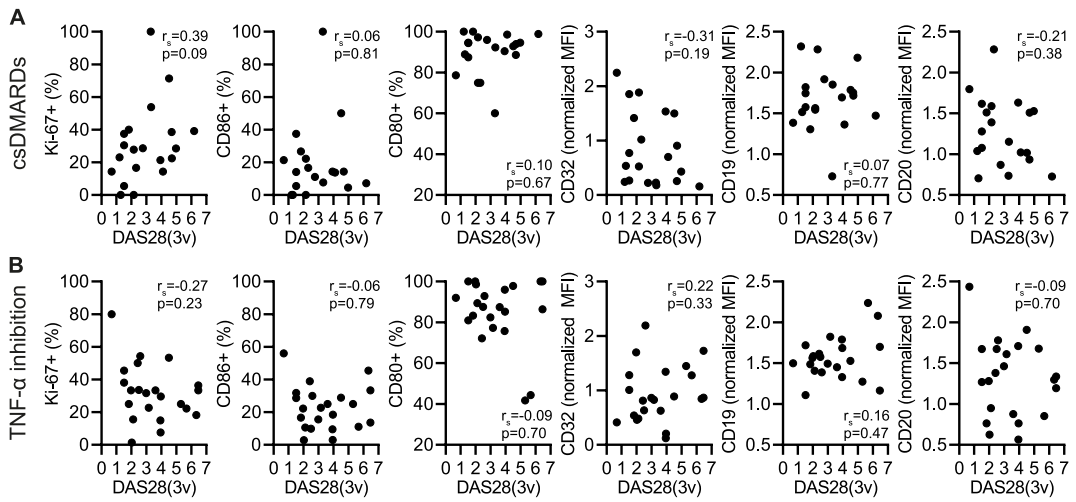

Fig. 3. Expression of activation markers by ACPA$^+$ MBCs in relation to clinical disease activity stratified by treatment.
(A–B) Correlation between the expression of Ki-67, CD86, CD80, CD32, CD19, CD20 by ACPA$^+$ MBCs and DAS28(3v) in a cross-sectional cohort of RA patients treated with csDMARDs (A) and TNF-α inhibitors. Median fluorescence intensities (MFIs) were normalized to MFIs expressed on non-antigen-specific (ACPA$^-$/TT$^-$) CD20$^+$CD27$^-$ B cells (naive). Correlations were described using Spearman's rank correlation coefficient (r_s).

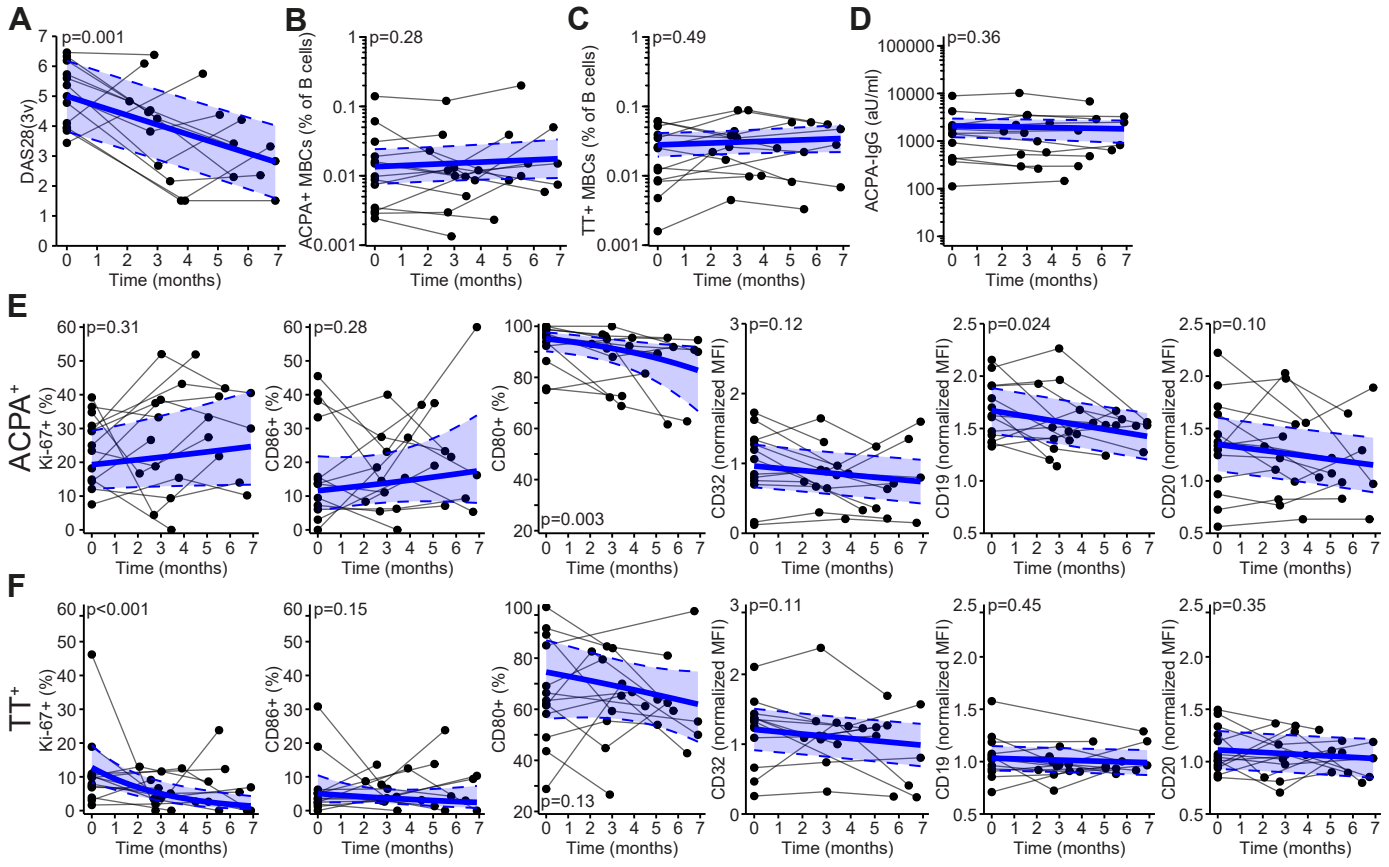

Fig. 4. Expression of activation markers by ACPA$^+$ MBCs over time in RA patients treated with JAK inhibitors. (A) Clinical response to treatment with JAK inhibitors as assessed by DAS28(3v). **(B–C)** Frequency of ACPA$^+$ (B) and TT$^+$ MBCs (C) as percentage of total B cells over time. **(D)** Circulating ACPA-IgG levels expressed in arbitrary units per ml (aU/ml). **(E–F)** Expression of Ki-67, CD86, CD80, CD32, CD19 and CD20 by ACPA$^+$ MBCs (E) and TT$^+$ MBCs (F) upon initiation with JAK inhibitors. Median fluorescence intensities (MFIs) were normalized to MFIs expressed on non-antigen-specific (ACPA$^-$/TT$^-$) CD20$^+$CD27$^-$ B cells (naive). (A–F) Effects over time were assessed with linear mixed (continuous outcomes) or beta regression (percentages as outcomes) models. Solid blue line = modelled outcome, dashed line = 95 % confidence interval. $P < 0.05$ is considered statistically significant.

of activation markers by ACPA$^+$ MBCs fluctuated over time, especially in comparison to TT$^+$ MBCs (Fig. 4E and F). However, for Ki-67, CD86, CD32 and CD20, no consistent changes in expression levels were observed. The expression of CD80 and CD19 significantly decreased over time, but this effect was variable between patients and did not reach the levels of resting TT$^+$ MBCs within the examined time frame. In

conclusion, as for csDMARDs and TNF-α inhibition, therapeutic inhibition of JAK-dependent signaling did not revert the activation of ACPA$^+$ MBCs towards a resting state, at least during the first seven months of treatment.

4. Discussion

Reversal of chronicity of disease is a major, unresolved challenge in the management of RA and other systemic AIDs. Here, we studied the disease-specific, autoreactive B cell response against citrullinated protein antigens and show that this response is highly active in patients with established RA, irrespective of clinical disease control with various therapeutic agents.

The continued presence of an activated ACPA B cell response indicates chronic exposure of these cells to stimulating triggers. This contrasts B cell responses to vaccinations or acute infections which show similar activation upon antigen encounter yet gradually revert to a resting MBC state over time [9,10]. TT$^+$ MBCs, for example, remained inactive throughout our analyses despite various degrees of systemic inflammation. ACPAs recognize a variety of citrullinated proteins and proteins with other post-translational modifications (PTM), like carbamylated and acetylated antigens [25,26]. So far, it is unknown whether specific PTM-antigens drive the ACPA MBC response, and whether these comprise autoantigens only or also foreign PTM-antigens at e.g. mucosal surfaces. Consequently, also the location and context in which these cells respond to PTM-antigens is not entirely clear. Yet, the observation that ACPA$^+$ MBC are less activated in the phase of clinical suspect arthralgia (CSA) [17] suggests that certain triggers may be present and persist in established RA that are (still) absent in CSA.

Irrespective of the nature of the stimulating trigger, however, our data suggest persistence of uncontrolled immunologically active disease despite anti-inflammatory treatment. It is conceivable that ACPA$^+$ MBCs contribute to inflammation by persistently providing co-stimulation to T cells and the production of pro-inflammatory cytokines, such as IL-6, IL-8 and TNF-α [17]. Activated circulating cells might migrate towards synovial tissue and eventually differentiate locally, as ACPA$^+$ PBs/PCs are enriched in inflamed synovium of patients [17]. In this context, it is remarkable that the ACPA$^+$ B cell response does not seem to be affected by various anti-inflammatory treatments described to suppress crucial B cell activation and differentiation pathways, like TNF-α and JAK inhibitors [24,27–29]. These treatments do, for example, inhibit B cell proliferation in vitro and impair B cell responses to vaccination [30–32]. This suggests that ACPA$^+$ B cells do not depend on these pathways to the same extent as non-autoreactive B cells, and that different mechanisms may be operational that allow these cells to persist. The resistance of ACPA$^+$ B cells to the investigated therapeutics might relate to the aberrant signaling of B cells observed in autoimmune diseases [33]. In this study, for example, we observed a marked decrease in the expression of the inhibitory receptor CD32 by ACPA$^+$ B cells. It is possible that ACPA$^+$ B cells are less sensitive to regulatory signals and more prone to stimulation. The latter is supported by the effects of glycans that are abundantly present in the variable domain of ACPA B cell receptors, which lower the threshold for ACPA B cell activation [34]. More detailed studies are required to study the mechanisms underlying the derailed activation of autoreactive B cells.

Together, the resistance of ACPA$^+$ MBCs to revert to a resting phenotype, also in the presence of these treatments, may be an important chronicity factor of RA and could explain disease flares upon drug tapering. The continuous activation of autoreactive B cells is conceivably present in other AIDs as well. Understanding the persistence and dynamics of these B cell responses may therefore identify therapeutic targets that allow for silencing and thereby cessation of the chronicity of human AIDs.

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CRediT authorship contribution statement

Sam Neppelenbroek: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Nienke J. Blomberg:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Arieke S.B. Kampstra:** Writing – review & editing, Methodology, Investigation. **Joost G. K. van der Hem:** Writing – review & editing, Formal analysis. **Tom W.J. Huizinga:** Writing – review & editing, Conceptualization. **René E.M. Toes:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Hans U. Scherer:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hans Ulrich Scherer reports financial support was provided by Pfizer Inc (ASPIRE program, grant ID 53248693) and Lilly Netherlands (protocol I4V-NS-O018). Hans Ulrich Scherer reports a relationship with Lilly Netherlands that includes: speaking and lecture fees. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2024.103320.

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