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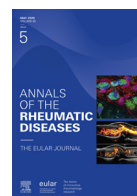
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Rheumatoid arthritis

Dimerization as a key feature of autoreactive IgA antibody responses

Anouk G. van Mourik¹, Jim B.D. Keijser^{2,3,4}, Myrthe A.M. van Delft^{3,4},
Sanne Reijm⁵, Alice Bacon¹, Theo Rispens^{2,3,4}, Nivine E.W. Levarht¹,
Rayman T.N. Tjokrodirjo⁶, Peter A. van Veelen⁶, Leendert A. Trouw⁷,
René E.M. Toes¹, Karin A. van Schie¹, Nienke Oskam^{2,3,4},
Diane van der Woude^{1,*}, T2B! immunity against SARS-CoV-2 study group

¹ Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands

² Sanquin Research Amsterdam, Amsterdam, The Netherlands

³ Department of Molecular Cell Biology and Immunology, Amsterdam UMC location Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

⁴ Amsterdam Institute for Immunology and Infectious Diseases, Immunology, Amsterdam, The Netherlands

⁵ Department of Immunology, MS Center ErasMS, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

⁶ Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands

⁷ Department of Immunology, Leiden University Medical Center, Leiden, The Netherlands

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ABSTRACT

Objectives: The presence of immunoglobulin A (IgA) autoantibodies has been described in many autoimmune diseases, and some of its characteristics, such as IgA dimerization, are considered a sign of a mucosal origin. However, limited information is available about the (patho)physiological conditions leading to the development of monomeric vs dimeric (autoreactive) IgA in humans. Therefore, we investigated IgA dimerization in rheumatic autoimmune diseases with a possible mucosal origin, as well as after vaccination.

Methods: Plasma of patients with rheumatic disease (rheumatoid arthritis [RA], systemic sclerosis [SSc], anti-neutrophil cytoplasmic antibody associated vasculitis [AAV], systemic lupus erythematosus [SLE]), SARS-CoV-2 vaccinated healthy individuals, and healthy controls was used for size exclusion chromatography (SEC). Enzyme-linked immunosorbent assays were performed on SEC fractions to determine the size of IgA. Results were confirmed using western blot and tandem mass spectrometry.

Results: The proportion of dimeric IgA was increased for autoantibodies compared to total IgA. This was most evident in RA, AAV, and SLE (dimeric autoreactive IgA $\approx$ 60%–80% vs total IgA $\approx$ 20%, SLE $\approx$ 60% total IgA), but not in SSc. Intramuscular vaccination against SARS-CoV-2 also led to an increased proportion of dimeric anti-spike IgA shortly after vaccination, irrespective of previous mucosal exposure.

Conclusions: These findings indicate that dimeric (autoreactive) IgA responses are associated with newly emerging antigen-specific immune activation, where production temporarily shifts to dimeric IgA. Mucosal triggering is not necessarily always involved in these IgA immune responses. These findings provide key insights into the circumstances for IgA dimerization and suggest that dimeric IgA could serve as a marker for immunological disease activity in autoimmunity.

*Correspondence to Dr. Diane van der Woude, Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands.

E-mail address: dvanderwoude@lumc.nl (D. van der Woude).

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T2B! immunity against SARS-CoV-2 study group members are listed in [Supplementary Material](#).

WHAT IS ALREADY KNOWN ON THIS TOPIC

- Immunoglobulin A (IgA) and especially dimeric IgA play a crucial role in mucosal and vaccine responses.
- Mucosal triggers have been hypothesized to underlie the emergence of autoreactivity in various rheumatic diseases.

WHAT THIS STUDY ADDS

- In several rheumatic diseases, autoantibody IgA is predominantly present in a dimeric state.
- Mucosal triggering is not necessarily always involved in the generation of dimeric IgA.
- Dimerization of IgA can be regulated by recent immune activation, shifting the IgA production temporarily to dimeric IgA.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- Dimeric IgA could serve as a marker for immunological disease activity in rheumatic autoimmune diseases such as rheumatoid arthritis.

INTRODUCTION

Immunoglobulin A (IgA) is a key mediator in immune protection, and especially plays an important role in neutralizing antigens. Recently, it became apparent that IgA is important in COVID-19 vaccine responses, where it is superior in neutralizing antigens compared to IgG, and where the size of IgA determines the efficiency of neutralization [1,2]. Previously, a similar mechanism has been described in influenza [3,4]. In addition to its neutralizing function in vaccine responses, IgA helps maintain homeostasis with foreign commensal microbes at mucosal sites. The size of IgA is key in the functions it exhibits, and differences can be observed between the composition of IgA in circulation and in mucosal secretions. In circulation, IgA is mainly found in the monomeric form, while a small amount is dimeric, composed of two IgA molecules and a joining (J) chain [5–7]. Dimeric IgA can be translocated across the epithelium from the basolateral surface to the apical surface via the polymeric immunoglobulin receptor (pIgR). At the apical side, the pIgR is cleaved, resulting in secretory IgA, which contains the secretory component (SC, a polypeptide derived from the pIgR) and the J chain. Since high amounts of dimeric IgA are encountered at mucosal sites, dimeric IgA has long been linked to local mucosal antibody production [8].

Mucosal triggers and local mucosal immune responses are believed to play a role in certain autoimmune responses [9]. These diseases, including rheumatoid arthritis (RA), systemic sclerosis (SSc), anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV), and systemic lupus erythematosus (SLE), are characterised by autoantibodies. These autoantibodies have provided important insights into the underlying immunological mechanisms, as evidenced by their association with key genetic risk factors [10,11]. This applies to autoantibodies in RA directed against post-translational modifications (PTMs), mainly anti-citrullinated protein antibodies (ACPA), as well as to ANCA in AAV [12–14]. While it is unknown if there is a pathogenic role for these autoantibodies, they serve as prominent markers for the underlying disease [15,16].

IgA autoantibodies are present in many rheumatic diseases [17–20]. In RA, ACPA of the IgA isotype can be detected years before disease onset [17]. This is in line with the concept that the induction of autoantibody responses occurs after mucosal

triggering, by, e.g. a bacterial protein harbouring a PTM, that elicits an immune response cross-reactive to modified self-proteins, ultimately leading to the development of RA [9]. There are several other indications that a mucosal site might be associated with the development of RA. For example, ACPA and rheumatoid factor (RF) IgA have not only been detected in blood but also in mucosal secretions such as sputum [21,22]. Furthermore, citrullinated bacterial antigens can be detected at mucosal sites and bound by human ACPA *in vitro* [23].

As limited information is available about the circumstances leading to the formation of dimeric IgA antibodies, and as insights into this aspect could indicate that the mucosa may be the site for breach of tolerance in rheumatic autoimmune diseases, we set out to characterise the IgA response in RA, AAV, SLE, and SSc. To investigate the occurrence of dimer formation, we also explored the dynamics of a *de novo* IgA response to SARS-CoV-2 induced after intramuscular vaccination or after infection.

METHODS*Patient and control samples*

Peripheral blood samples of 12 patients with ACPA+ RA, 11 anti-topoisomerase (ATA) positive SSc, and 12 anti-myeloperoxidase (MPO) positive patients with AAV and 6 patients with anti-double-stranded(ds)-DNA-positive SLE visiting the outpatient clinic of the rheumatology department at the Leiden University Medical Center (LUMC) were included in this study. Additionally, 3 healthy control (HC) samples and 3 patients with ACPA- RA were also included. The patient characteristics are reported in [Supplementary Material](#) (disease, year of sampling, age, sex (male (M) & female (F)), disease duration at the point of sampling in years, disease activity [for RA and SLE], organ involvement [for SSc], diagnosis [for AAV], treatment and B cell-depleting therapies [only reported here for AAV]). All other participants had no B cell-depleting therapies before sampling. For SARS-CoV-2 vaccinated individuals, peripheral blood samples of 9 HC participants were obtained from the previously described Target-2-B! cohort study, including 3 participants who were SARS-CoV-2-naïve before vaccination and 6 who were experienced as a result of previous infection ([Supplementary Material](#)).

Study approval

Written informed consent was obtained from all individuals with RA, ANCA, SSc, SLE, and HC, and all protocols were approved by the ethical committee of the LUMC, the Netherlands. Regarding the samples from the T2B! immunity against SARS-CoV-2 study group, this was approved by the medical ethical committee (NL74974.018.20 and EudraCT 2021-001102-30). All participants provided written informed consent. Patients or the public were not involved in the design of the study.

Antibody measurement by ELISA

In-house enzyme-linked immunosorbent assays (ELISAs) were performed to screen for the presence of autoantibodies. For the RA samples, ELISAs were carried out to measure RF IgA, anti-modified protein antibodies (AMPA) (ACPA, anti-carbamylated protein antibodies [anti-CarP], and anti-acetylated protein antibodies [AAPA]) IgA as described before with minor changes

reported in [Supplementary Material](#) [21]. For the AAV samples, the presence of anti-MPO IgA in serum was measured as described previously [24]. In short, Corning 384-well microplates (3700, Sigma Aldrich) were coated with human MPO (MY862, Elastin Products Company). Samples were tested at a 1:200 dilution for screening and a 1:2 dilution for size exclusion chromatography (SEC). Antibody binding was detected using goat anti-human IgA (1:500, 2050-04, Southern Biotech) followed by visualisation with p-nitrophenyl phosphate (34047 and 36064, Sigma) at 405 nm. For SSs, an ATA IgA ELISA was performed as described before with minor changes (adapted from [18]). Lastly, for SLE, an anti-dsDNA IgA ELISA was performed. Corning 384-well microplates (3700, Sigma Aldrich) were coated with ultraPure Salmon Sperm DNA Solution (15632011, ThermoFisher). Samples were tested in a titration starting at 1:10 for sera (screening) and 1:2 dilution after SEC. Antibody binding was detected using goat anti-human IgA horseradish peroxidase (HRP) (A80-102P, Bethyl). SEC fractions of rheumatic diseases and HC were also utilised in total Ig ELISAs for IgM (1:250 or 1:500), IgA (1:3000 or 1:5000), and IgG (1:5000) (A80-100, 102, and 104, Bethyl Laboratories) according to the manufacturer's protocols. All ELISAs were developed using ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid, in-house) and measured at 415 nm.

For the detection of SARS-CoV-2-specific antibodies, 96-well maxisorp plates (ThermoFisher) were coated overnight with recombinant Wuhan-Hu-1 (WH1) full spike (1 µg/mL). Fractionated sera were tested at 1:50 and 1:500 dilutions in phosphate-buffered saline (PBS) with 0.1% v/v Tween-20 and 0.2% w/v gelatin (PTG buffer) and bound IgA was detected with mouse anti-human IgA HRP (0.5 µg/mL, MH-14-1-HRP, Sanquin) in PTG buffer. A serum pool with a known concentration of IgA was used as a reference. Total IgA was measured similarly, using mouse anti-human IgA (1 µg/mL, MH14-1, Sanquin) as coating instead, and samples were tested at 1:1000 and 1:5000 dilution. Binding was visualised using tetramethylbenzidine, and the absorbance was read at 450 nm and 540 nm for background correction.

Size exclusion chromatography

Plasma of participants was diluted 1:1 in PBS, 1:3 for SARS-CoV-2 vaccinated participants, and subsequently filtered (0.2 µm filters, Millipore). SEC of the plasma of rheumatic diseases and HC was performed using the ÄKTA Pure equipped with a Hiloal 16/600 Superdex 200 column (GE Healthcare, 28-9893-35) and analyzed with Unicorn software (version 7.1) according to the manufacturer. The SARS-CoV-2 sera were run on a Superdex 200 10/300 GL column (GE Healthcare) using an ÄKTA Go system.

Western blot

For SDS-PAGE, plasma from a patient with RA (1:2000 diluted), purified human IgA (20 ng) and purified AMPA (800–1000 ng; containing AMPA IgG, IgM and IgA, purified according to [25]), dimeric human IgA (666x diluted, Nordic), and plasma of an IgA-deficient donor (20 µL) were mixed with Laemmli buffer (Bio-Rad) and incubated for 5 min at 95°C. Samples were loaded on a 4% to 15% mini TGX precast protein gel (Bio-Rad), and the PageRuler™ Plus Prestained Protein Ladder (Thermo Fisher) was used as reference. Proteins were blotted on a mini 0.2 µm polyvinylidene fluoride (PVDF) membrane using the Trans-blot Turbo system (Bio-Rad). Blots were blocked with PTS

(PBS/0.05% Tween-20/3% skim milk powder [Sigma]) at 4°C overnight. The next day, primary staining was performed at RT with polyclonal rabbit anti-human IgA (1:10,000 in PTS, DAKO, A0262) and afterwards at RT with polyclonal goat anti-rabbit-Ig HRP (1:5000 in PTS, DAKO, P0448) in PTS. Blots were developed using Pierce ECL Western Blotting Substrate (GE Healthcare), the readout was performed on a Bio-Rad Chemidoc Touch Imaging system, and the images were analyzed using Image Lab (Version 6.1).

Tandem mass spectrometry (MS/MS)

In short, tandem mass spectrometry (MS/MS) was performed on 3 size exclusion fractions (A, B, and C) of 3 patients to analyse the presence of J chain and SC. Approximately 1 µg of IgA was purified from these fractions by a 2-hour incubation with CaptureSelect IgA Affinity Matrix beads (Thermo Scientific, 1942880250) on RT. IgA was released from the beads by incubating them in Laemmli buffer at 95°C for 5 minutes. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as previously described and stained with Coomassie blue (Abcam) for 15 minutes. For MS analysis, gel bands of interest (ie, monomeric [A], dimeric [B], and polymeric [C] IgA) were cut out and processed as described in [Supplementary Material](#).

Statistical analysis

For ELISA, arbitrary units (AUs) were calculated from the standards that were added to all ELISAs. For graphs, AUs were normalized to a percentage whereby the highest AU in each participant for a particular ELISA was set to 100%. To calculate the percentage of high molecular weight (HMW) IgA, we defined a cut-off distinguishing monomeric from HMW IgA for each individual participant, using the area under the curve of each respective ELISA. As illustrated in [Figure 1E](#), the cut-off was set 2 fractions to the left based on the highest monomeric peak of the total IgA. Statistical analysis was performed using GraphPad Prism 7.02. Kruskal-Wallis tests were performed to investigate differences in the percentage of HMW IgA. A Pearson correlation was performed to investigate the differences between clinical parameters and the proportion of HMW IgA. *P* values below .05 were considered statistically significant. Significant differences are noted as follows: not significant (ns; *P* > .05), **P* < .05, ***P* < .01, ****P* < .001, or *****P* < .0001.

RESULTS

Characterization of IgA autoantibodies in RA

To investigate the size distribution of IgA autoantibodies in patients with RA, plasma was fractionated using SEC. Fractions were collected and analyzed for the presence of antibodies by total Ig ELISAs in patients with ACPA+ RA, ACPA− RA, and HC ([Fig 1A](#), [Supplementary Material](#)). No difference was detected between the size profiles of total IgM, IgA, or IgG in the patients with ACPA+ RA compared to the patients with HC or ACPA− RA ([Fig 1B](#), [Supplementary Material](#)). As expected, antibodies eluted according to size, with IgM eluting first, followed by IgA and lastly IgG. IgA eluted in a broad range of fractions, suggesting the presence of both monomeric and HMW IgA, although monomeric IgA dominated the SEC spectrum. Next, the size distribution of AMPA IgA and RF IgA in the fractions was investigated. All AMPA IgA, as well as RF IgA, were found to have a

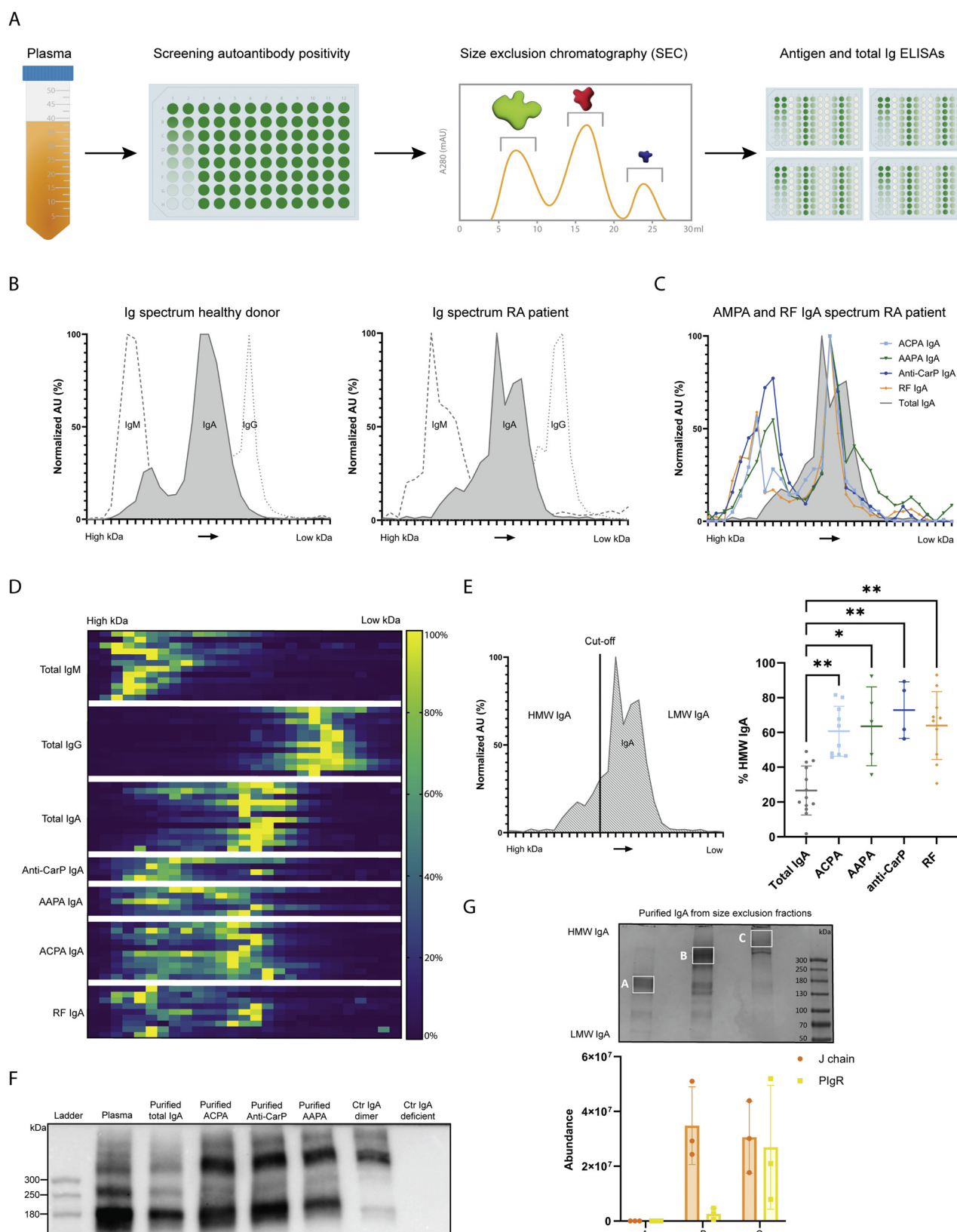


Figure 1. Characterization of IgA autoantibodies in RA. (A) Method for the determination of HMW IgA. Plasma samples of patients with RA were screened for AMPA positivity, and SEC followed by ELISA was conducted with positive samples. (B) Normalized AUs of total Ig patterns of a HC and a patient with RA (representative of $N = 3$). (C) AMPA and RF IgA compared to total IgA in a patient with RA positive for all AMPA (ACPA = light blue = square, AAPA = green = triangle, anti-CarP = dark blue = circle) and RF (RF = orange = diamond) IgA. (D) Heatmap of the ELISA data of SEC fractions of 12 patients with RA (anti-CarP $N = 4$, AAPA $N = 5$, ACPA $N = 10$, RF $N = 9$). (E) Cut-off for HMW IgA and LMW IgA based on the total IgA peak of each participant. The percentages of HMW IgA are depicted in the graph for all the participants with RA. A nonparametric test, Kruskal-Wallis test, was performed to compare total IgA to ACPA IgA ($P < .01$), AAPA IgA ($P < .05$), anti-CarP IgA ($P < .01$), and RF IgA ($P < .01$). (F) IgA western blot of plasma, purified plasma IgA, purified AMPA of a patient with RA, commercial dimeric IgA antibody (control), and IgA-deficient serum (control). (G) SDS-Page of 3 different fractions from the SEC of a patient with RA (representative of $N = 3$). The respective bands visible in the SDS-Page were subjected to MS/MS. The summed abundance of 3 peptides in, respectively, J chain (J chain = orange = circle and 3 peptides in SC (SC = yellow = square) is depicted in the graph for 3 patients with RA for each band

larger proportion of HMW species compared to total IgA (Fig 1C). We confirmed this change in size distribution towards HMW of autoreactive IgA in other patients with ACPA + RA for all the AMPA and RF IgA (Fig 1D). The proportion of HMW autoantibody IgA was different for each patient, in some cases resulting in a complete shift of the peak from monomeric autoantibody IgA towards the HMW protein side. The percentage of HMW IgA was significantly higher in ACPA IgA ($P < .01$), AAPA IgA ($P < .05$), anti-CarP IgA ($P < .01$), and RF IgA ($P < .01$) compared to total IgA (Fig 1E). The increased proportion of dimeric ACPA IgA also demonstrated a correlation ($R = 0.47$) with the disease activity score, although not significant. An inverse correlation was seen between the symptom duration and the proportion of dimeric ACPA IgA ($R = -0.50$, NS) (Supplementary Material). These data complement each other since disease activity generally decreases with a longer disease duration. Age did not show any association with the proportion of dimeric autoantibody IgA.

To confirm whether this change in size distribution could be attributed to the presence of HMW IgA, we subsequently isolated AMPA and total IgA. Isolated supernatant was next submitted to SDS-PAGE, and the molecular weight of purified AMPA and total purified IgA from the same ACPA + participant was assessed by IgA-specific western blot (Fig 1F). Total plasma as well as purified IgA from plasma demonstrated a band at the expected height of monomeric IgA. For AMPA IgA, a second band, with the same intensity as the monomer band, was visible at the height of a control IgA dimer. This band can only be explained by interactions via disulfide bridges, such as J chain binding to IgA, indicating the abundant presence of HMW IgA in the isolated IgA AMPA fractions. To further investigate whether the difference in IgA distribution, detected after SEC, was indeed due to the formation of HMW IgA, we utilized MS/MS. The abundance of J chain and SC was analyzed in 3 purified IgA SEC fractions (A, B, and C) of participants with ACPA + RA by MS/MS (Fig 1G). No sequences derived from J chain or SC were detected in the monomeric fractions (band A). In contrast, in band B at the height of dimeric IgA, J chain-derived sequences were detected, indicating the presence of J chain. In the polymeric fraction, band C, sequences derived from J chain as well as SC were present. Although we also detected the presence of IgM, this was at a considerably lower abundance as compared to IgA, indicating that the J chain and SC were mainly associated with IgA. Together, these data indicate that AMPA IgA are present in a dimeric form, as shown by the increased size and the presence of J chain and SC.

The size of IgA autoantibodies in rheumatic autoimmune diseases

To address the question of whether the increase in the proportion of dimeric autoreactive IgA is specific for RA, we next investigated IgA autoantibodies in other autoimmune rheumatic diseases. To this end, SEC was performed on plasma samples from patients with AAV, SSc, and SLE followed by the measurement of total Ig and the respective autoantibody IgA by ELISA. The analysis of sera from patients with AAV revealed changes in the distribution of anti-MPO IgA compared to total IgA, in line with the results of patients with RA ($P < .0001$)

(Fig 2A). Participants with SLE also demonstrated an increased proportion of dimeric anti-dsDNA IgA compared to total IgA ($P < .01$). Surprisingly, total IgA also had an increased proportion of dimeric IgA in SLE compared to other rheumatic diseases (Fig 2C). Lastly, a more diffuse pattern was obtained by investigating the ATA response present in patients with SSc where in some cases a small proportion of dimeric ATA IgA was observed, whereas in other cases almost no dimeric ATA IgA was detectable (Fig 2B). Together, these data indicate that an increase in dimeric autoantibody IgA is found in some but not all autoimmune diseases.

HMW IgA formation upon vaccination

As dimeric IgA responses in rheumatic diseases are hypothesized to be related to a mucosal trigger, we aimed to investigate the dynamics of mucosal and non-mucosal immune response by taking primary (intramuscular vaccination) and recall (respiratory infection) responses in SARS-CoV-2 as a model [9]. To this end, we examined serum samples from individuals who developed an immune response to SARS-CoV-2 through infection before vaccination and from individuals without such exposure before vaccination ('Naïve'). Samples were included from 4 different timepoints: directly before the first vaccination (Pre-Vac 1), 10 days after the first vaccination (Post-Vac 1), directly before the second vaccination (Pre-Vac 2), and 10 days after the second vaccination (Post-Vac 2) (Fig 3A). As shown in Figure 3B, the vaccinations elicited a SARS-CoV-2-specific immune response with detectable anti-spike IgA levels. Samples before vaccination 1 were negative for anti-spike IgA in all naïve individuals and 3 out of 6 previously infected individuals ('Nondetectable Pre-Vac 1'). The other 3 previously infected individuals had positive anti-spike IgA levels at this timepoint and had reported positive SARS-CoV-2 polymerase chain reaction (PCR) tests 22, 38, and 55 days before their first vaccine dose and were therefore analyzed as a separate subset ('Detectable Pre-Vac 1'). The other 3 individuals, 'Nondetectable Pre-Vac 1', were positive for anti-nucleocapsid antibodies or had a positive PCR test more than half a year before the study.

We then investigated the dynamics of dimeric anti-spike IgA after primary or recall responses by SEC. Unsurprisingly, the 'Detectable Pre-Vac 1' group showed dimeric anti-spike IgA in all the sampled timepoints, with the largest proportion of dimeric IgA at Post-Vac 1 (Fig 3C, Supplementary Material). In the 'Nondetectable Pre-Vac 1' group, the anti-spike IgA response after Post-Vac 1 also clearly demonstrated a highly dimeric nature. Later timepoints showed less dimeric IgA, indicating that repeated vaccination in a short time period did not lead to, or sustain, a markedly dimeric anti-spike IgA response. Surprisingly, in the naïve group, the intramuscular COVID vaccination also led to an anti-spike IgA response with dimeric IgA. An increase in dimeric IgA was visible compared to total IgA for the 2 timepoints at which anti-spike IgA could be detected.

DISCUSSION

In this study, we characterised the dimerization of the IgA autoantibody response in rheumatic autoimmune diseases. The

separately (A, B, and C). AAPA, anti-acetylated protein antibody; ACPA, anti-citrullinated antibody; AMPA, anti-modified protein antibodies; anti-CarP, anti-carbamylated protein antibodies; AU, arbitrary unit; ELISA, enzyme-linked immunosorbent assay; HC, healthy control; HMW, high molecular weight; Ig, immunoglobulin; LMW, low molecular weight; MS/MS, tandem mass spectrometry; SC, secretory component; SDS-Page, sodium dodecyl-sulfate polyacrylamide gel electrophoresis; PIgR, polymeric immunoglobulin receptor; RA, rheumatoid arthritis; RF, rheumatoid factor; SEC, size exclusion chromatography.

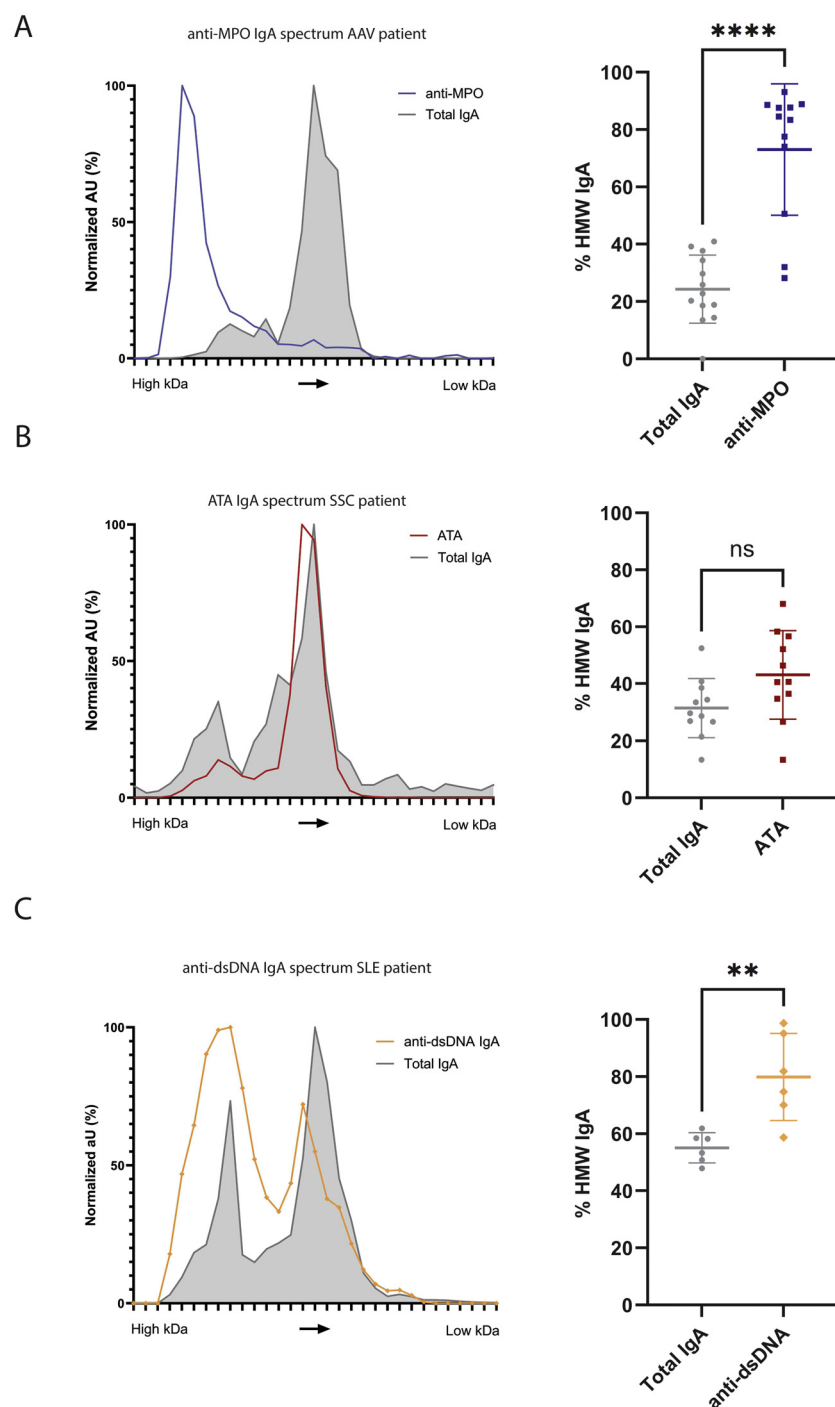


Figure 2. The size of IgA autoantibodies in AAV, SSc, and SLE. (A) Left is the SEC profile for a patient with AAV (representative of $N = 12$). The normalized AU values are shown for the respective autoantibody (anti-MPO IgA = purple square) as well as for total IgA. Right is the percentage HMW IgA for the autoantibody and total IgA. A nonparametric test, Kruskal-Wallis test, was performed (anti-MPO [$P < .0001$]). (B) Left is the SEC profile for a patient with SSc (representative of $N = 11$). The normalized AU values are shown for the respective autoantibody (ATA IgA = red square) as well as for total IgA. Right is the percentage HMW IgA for the autoantibody and total IgA. A nonparametric test, Kruskal-Wallis test, was performed (ATA = ns). (C) Left is the SEC profile for a patient with SLE (representative of $N = 6$). The normalized AU values are shown for the respective autoantibody (anti-dsDNA IgA = orange diamond) as well as for total IgA. Right is the percentage HMW IgA for the autoantibody and total IgA. A nonparametric test, Kruskal-Wallis test, was performed (anti-dsDNA IgA [$P < .01$]). AAV, ANCA-associated vasculitis; ATA, antitopoisomerase; AU, arbitrary unit; dsDNA, anti-double stranded DNA; HMW, high molecular weight; Ig, immunoglobulin; MPO, myeloperoxidase; ns, not significant; SEC, size exclusion chromatography; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

results revealed that the RA-associated AMPA and RF IgA responses had an increase in dimeric IgA compared to total IgA, which consisted of approximately 20% dimeric IgA, while AMPA and RF consisted of 60% to 80% dimeric IgA. This was not exclusive to RA autoantibodies as the AAV-associated anti-MPO response as well as the anti-dsDNA response in SLE displayed similar features, indicating that increased dimeric IgA responses are present in other, although not all, autoimmune diseases. Surprisingly, the proportion of dimeric total IgA in SLE was increased compared to other autoimmune diseases. A possible explanation could be that the multitude of different autoantibodies known to be present in SLE, this could affect the total proportion of dimeric total IgA if they make up a significant amount of the total IgA [20,26]. Furthermore, to shed light on the origin of this dimeric autoimmune response, analysis of

plasma from healthy individuals vaccinated against SARS-CoV-2 revealed that anti-spike IgA displayed a similar predominance of dimeric IgA. As this was also observed in non-infected, but intramuscularly vaccinated individuals, these data reveal a crucial finding that mucosal immune activation is not required for the formation of a dimeric IgA response.

These outcomes are in line with previous studies indicating that elevated SC binding is observed to ACPA IgA, RF IgA, and total IgA in RA [27–29]. Likewise, our findings are in line with studies into post-vaccination IgA responses in patients with IgA nephropathy, where dimeric antigen-specific IgA increased shortly after vaccination [30,31]. Together with our new data on the presence of dimeric autoantibodies in relationship to clinical data and the dynamics of the IgA response after vaccination, a clear picture emerges indicating that dimeric IgA could be a

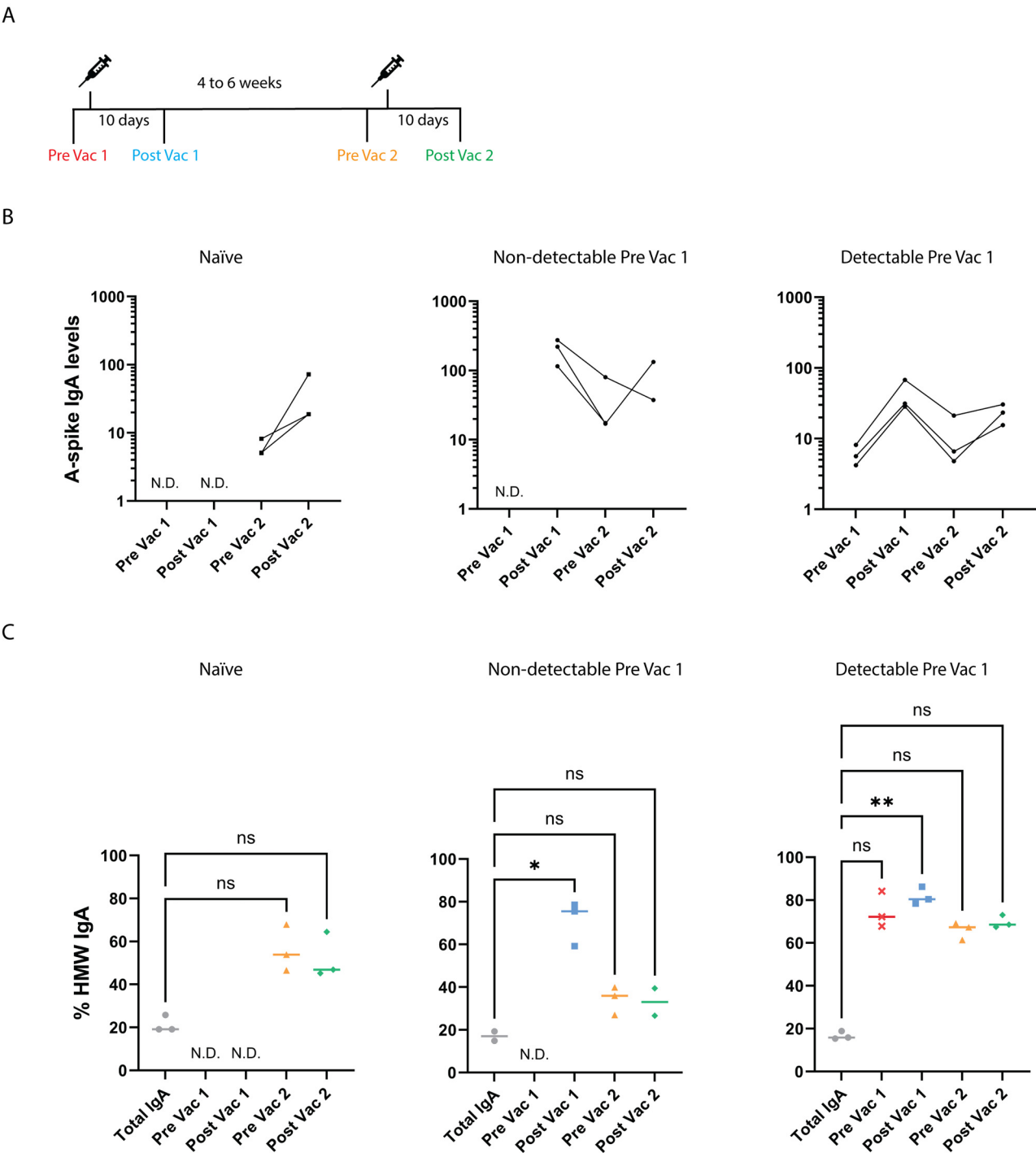


Figure 3. The dimeric IgA response after SARS-CoV-2 vaccination. (A) Scheme of the vaccination study. Samples were taken at 4 different timepoints (before vaccination 1 = Pre-Vac 1 = red/cross, 10 days after vaccination 1 = Post-Vac 1 = blue/square, before vaccination 2 = Pre-Vac 2 = orange/triangle, 10 days after vaccination 2 = Post-Vac 2 = green/diamond). Not all timepoints were available for all patients. Post-Vac 1 samples were not available for naïve participants, and Pre-Vac 1 samples were precluded from further analysis if seronegative in the screening anti-RBD IgA ELISA, which was the case in all 3 naïve participants and 3 of the previously infected individuals ('Nondetectable Pre-Vac 1'). (B) Anti-spike IgA levels at the different sample timepoints for the Naïve, Nondetectable Pre-Vac 1, and Detectable Pre-Vac 1 participants. N.D. = not determined. (C) Percentage of HMW IgA at different timepoints in SARS-CoV-2-vaccinated individuals. A nonparametric Kruskal-Wallis test was performed. Nondetectable Pre-Vac 1: Post-Vac 1 ($P < .05$). Detectable Pre-Vac 1: Post-Vac 1 ($P < .01$). Other timepoints were ns (not significant). ELISA, enzyme-linked immunosorbent assay; HMW, high molecular weight; Ig, immunoglobulin; anti-RBD, anti-receptor binding domain.

hallmark of recent immune activation. This can occur at mucosal surfaces, as evidenced by our data obtained following SARS-CoV-2 infection, but also at non-mucosal surfaces, as indicated by the observations made after intramuscular vaccination only. Nonetheless, it is still highly conceivable that the presence of dimeric autoreactive IgA results from immune activation at

mucosal sites as several lines of evidence implicate a mucosal contribution to the induction of autoreactive B cell-mediated immune responses [21,32,33]. Through the persistent presence of autoantigens, autoimmune responses could stay active for prolonged periods. This is in line with data regarding ACPA-producing B cells which are characterised by the expression of

activation and proliferation markers, like Ki-67, indicating their continuous activation [34,35]. These previous observations therefore parallel the findings in this study, as they also point to continuous immunological disease activity even in subjects receiving adequate treatment for RA.

An important question that remains is which signals lead to the development of this dimeric IgA response in B cells, which seems to be temporary. Different lineages of B cells might be responsible for the production of monomeric or dimeric IgA as there are differences in clonality between monomeric and dimeric IgA [6]. The amount of J chain, needed for the production of polymeric IgA, could also be a determining factor, although this seems less likely, considering that J-chain expression is known to be widespread in all B cells and even present in IgG-producing B cells, despite the fact that IgG only exists in monomeric form [36]. Whether J-chain chaperone-proteins, cytokine/chemokine mix, or B cell location influence dimeric IgA antibody production currently remains unclear [37]. Therefore, further research is needed to elucidate the underlying mechanism. The function of dimeric IgA in plasma is almost as elusive as the formation, although in SARS-CoV-2 and influenza, it has been suggested that dimeric IgA displays an increased neutralizing activity [1–4]. Hence, it is also interesting to further investigate the effects of dimeric IgA since it could also very well be hypothesized that the effector functions are proinflammatory, through, e.g. receptor binding and neutrophil activation [38]. Simultaneously, it would be important to perform more in-depth studies into a possible relationship between the occurrence of dimeric IgA autoantibody responses and disease activity and remission. In light of the findings, it appears plausible that there could be a relationship between disease duration as well as disease activity and dimeric autoantibody IgA formation. This would indicate that dimeric IgA could serve as a marker for immunological disease activity.

Our study has some limitations, as the sample size in some of the groups is relatively small. Likewise, our data do not allow definitive conclusions about the origin of dimeric autoreactive IgA or the exact mechanism regulating the production of dimeric IgA. However, the strength of this study is that we present a new concept regarding the dynamics of IgA responses. We have investigated this in 4 autoimmune diseases and a vaccination study of an infectious disease, whereby we confirmed our findings in ELISA with multiple different methods (ie, western blot and MS/MS) and gained valuable insight into the development of the dimeric IgA response.

In summary, we propose that dimeric IgA is a key feature of newly emerging and autoreactive immune responses. The size of IgA plays an important role in neutralization, making our findings very relevant for the development of efficient vaccines. Besides, dimeric IgA could also serve as a marker for immunological disease activity in autoimmunity. Consequently, our findings are key to understanding the dynamics of dimeric IgA responses in autoimmunity as well as in fundamental immunology.

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Contributors

AGvM designed the research studies, conducted experiments, acquired data, analyzed data, and wrote the manuscript. JBdK, SR, and AB conducted experiments, acquired data, and analyzed

data. NEWL conducted experiments. RT and PV acquired and analyzed data. MAMvD, LAT, and TR designed the study. REMT, KvS, NO, and DvdW designed the study and supervised the project. REMT, DvdW, and AGvM drafted the manuscript. T2B! immunity against SARS-CoV-2 study group provided samples. All authors provided critical feedback, reviewed the results, and approved the final version of the manuscript.

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Competing interests

REMT has a patent on anti-CarP in rheumatic diseases. TR is an inventor on a patent application based on the use of bioengineered IgG targets for the characterisation of rheumatoid factor reactivity patterns. The other authors declare they have no competing interests.

Patient consent for publication

Patients gave their written informed consent prior to their participation.

Ethics approval

The use of samples from the T2B! immunity against SARS-CoV-2 study group were approved by the medical ethical committee (NL74974.018.20 and EudraCT 2021-001102-30).

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Orcid

Anouk G. van Mourik: <http://orcid.org/0000-0002-3811-7373>

Diane van der Woude: <http://orcid.org/0000-0001-8121-5879>

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