Exploring the landscape of rheumatoid arthritis: piecing together risk factors and autoantibodies

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Autoantibody isotypes

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Letter

IgM antibodies against acetylated proteins as a possible starting point of the anti-modified protein antibody response in rheumatoid arthritis

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Rheumatoid arthritis (RA) is characterized by the presence of anti-modified protein antibodies (AMPA): anti-citrullinated protein antibodies (ACPA), anti-carbamylated protein antibodies (anti-CarP) and anti-acetylated protein antibodies (AAPA). These AMPA responses are specific for RA and have consistently been found to develop years before disease onset.[1, 2] Most studies on AMPA in (pre-disease) RA are focused on IgG antibodies. However, IgM is the first isotype generated in (auto)antibody responses. It is unclear whether IgM-autoimmunity differs between AMPA targeting different post-translational modifications (PTMs). Since this could provide relevant clues on the initiation of the AMPA response, we investigated IgM-levels of ACPA, anti-CarP and AAPA in different cohorts including healthy individuals, patients with RA and patients with non-RA arthritis consisting of patients with arthritis and rheumatic diseases other than RA (detailed descriptions of the cohorts and patient characteristics are provided in the supplementary material).

First, levels were determined in sera from the Nagasaki Island study[3] (supplementary material). High levels of ACPA-IgM and anti-CarP-IgM were found in a subgroup of the ACPA-IgG positive healthy donors (HD), but not in ACPA-IgG-negative HD or ACPA-IgG-negative non-RA patients (Figure 1A). Furthermore, high AAPA-IgM levels could be readily detected among all groups and no significant difference in AAPA-IgM levels was found between ACPA-IgG-negative and ACPA-IgG-positive HD. The same pattern was also seen in sera from the early arthritis clinic cohort (EAC),[4] showing high levels of ACPA-IgM and anti-CarP-IgM almost exclusively in ACPA-IgG-positive RA patients, but not in HD (figure 1C and table S4). Again, high AAPA-IgM levels were not only observed in ACPA-IgG-positive RA patients, but also in some of the HD, non-RA patients and ACPA-IgG negative RA patients (figure 1C). This distinct AAPA pattern in both cohorts was not observed for the IgG antibodies (figure 1B, 1D and table S4). In line with this notion, a moderate to strong correlation was found between IgG and IgM levels for ACPA and anti-CarP in both cohorts, whereas the correlation between AAPA-IgG and AAPA-IgM was weak/absent (figure S1A and S1C). Likewise, ACPA-IgM and Anti-CarP-IgM-levels showed a moderate/strong correlation in both cohorts, while the correlation between AAPA-IgM and levels of ACPA-IgM or anti-CarP-IgM was absent or weak (Figure S1B and S1D). To investigate whether AAPA-IgA might also be present in healthy individuals, this isotype was measured in the EAC cohort and healthy controls. High levels of AAPA-IgA were almost exclusively found in ACPA-IgG-positive RA patients, although a few healthy individuals also have detectable AAPA-IgA levels (Figure S2A). Moreover, the correlation between AAPA-IgA and AAPA-IgG was stronger than the correlation between AAPA-IgA and AAPA-IgM (Figure S2B), underlining the distinct pattern found in AAPA-IgM. The lack of reactivity to control peptides without PTMs confirmed that the AAPA IgM-signal was not due to non-specific binding (Figure S3). Inhibition

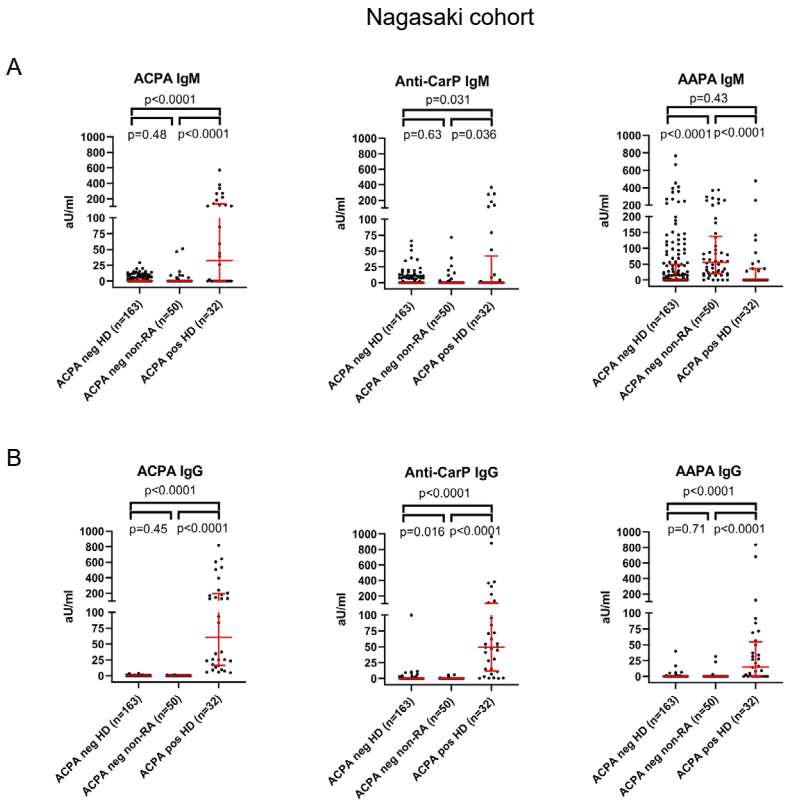

Figure 1. A) ACPA-IgM, Anti-CarP-IgM and AAPA-IgM levels in arbitrary units (aU) of Nagasaki Island cohort. B) ACPA-IgG, Anti-CarP-IgG and AAPA-IgG levels in aU of Nagasaki Island cohort. CCP4, CHcitP4 and CAcetylP4 were used as antigens. Medians with interquartile range are indicated in red. Signals from the control peptides CArgP4 and CLysP4 were subtracted from the CCP4 and CHCitP4 or CAcetylP4 signal, respectively.

experiments using different antigenic backbones provided further validation of the specificity of this response (supplementary material and Figure S4). Lastly, mixing experiments were performed that confirm that the measured AAPA-IgM signal was not due to binding of RF-IgM to AAPA-IgG (supplementary material and Figure S5). It has been shown at the polyclonal and monoclonal level that both AMPA-IgG and IgM can be highly cross-reactive.[5] While AAPA-IgG is restricted to ACPA-IgG-positive individuals, we show that AAPA-IgM is a more distinct response compared to the other AMPA.[6] In line with this data, not all AMPA-IgM are cross-reactive as a monoclonal AAPA-IgM antibody restricted to acetylated epitopes has been found previously.[5] Perhaps further maturation of the AMPA response from AAPA-IgM preferentially occurs in (a minority of) individuals in whom the AAPA-IgM response shows a tendency for cross-reactivity. A limitation of the current study is the absence of a cut-off for AAPA-

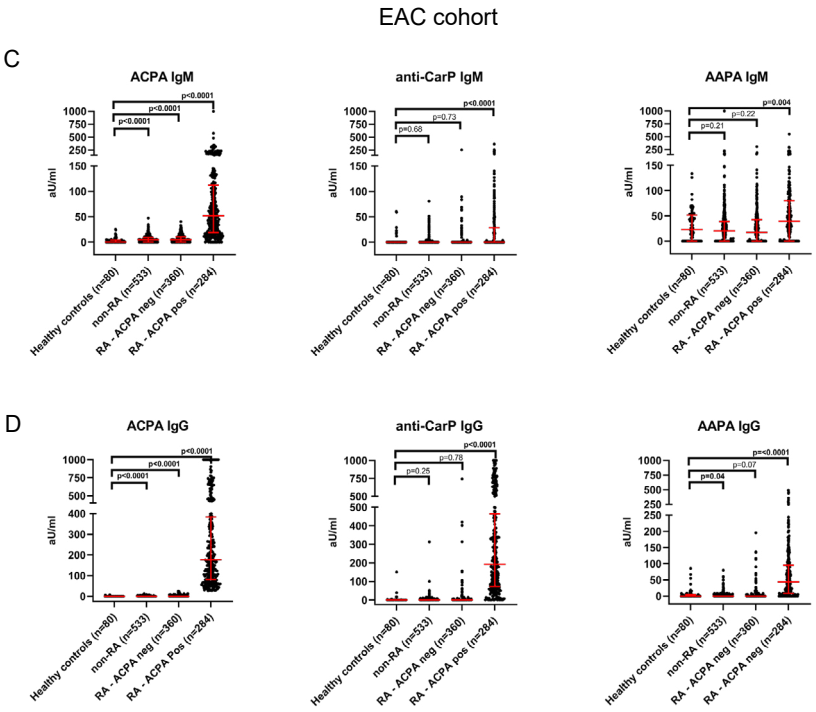

Figure 1. C) ACPA-IgM, Anti-CarP-IgM and AAPA-IgM levels in aU of the EAC cohort. D) ACPA-IgG, Anti-CarP-IgG and AAPA-IgG levels in aU of the EAC cohort. CCP2, CHcitP2 and CAcetylP2 were used as antigens. Medians with interquartile range are indicated in red. Signals from the control peptide CArgP2, ClysP2 and CnorleuP2 were subtracted from the CCP2, CHcitP2 and CAcetylP2 signal, respectively.
ACPA: anti-citrullinated protein antibodies, Anti-CarP: anti-carbamylated protein antibodies, AAPA: anti-acetylated protein antibodies, CCP4: anti-cyclic citrullinated peptide 4, CArgP4 anti-cyclic arginine peptide 4, CHcitP4: anti-cyclic homocitrullinated peptide 4, CLysP4: anti-cyclic lysine peptide 4, CAcetylP4: anti-cyclic acetylated peptide 4, EAC: early arthritis clinic, CCP2: anti-cyclic citrullinated peptide 2, CArgP2 anti-cyclic arginine peptide 2, CHcitP2: anti-cyclic homocitrullinated peptide 2, CArgP2 anti-cyclic lysine peptide 2, CAcetylP2: anti-cyclic acetylated peptide 2, CNleuP2: anti-cyclic norleucine peptide 2.

IgM positivity, which could not be calculated due to the high levels of AAPA-IgM in healthy controls. Strengths of the current study are the use of unique cohorts from different continents, allowing to investigate AMPA before disease and early in the disease, the inclusion of arthritis patients with other diagnoses than RA and the specific AMPA-assays based on the same antigenic backbone for all PTMs. In conclusion, our observations suggest that AAPA-IgM is part of the "normal" immune repertoire and might possibly constitute a starting point for RA-associated AMPA responses, after which isotype switching and epitope spreading to other PTMs could lead to the typical RA-associated AMPA response.

Supplementary material
Supplementary material available at:
https://www.sciencedirect.com/science/article/pii/S0003496724003534?via%3Dihub

Competing interests
None

Contributors
TJvW, SR, ALD and GS performed experiments. SR and TJvW analysed data and drafted the manuscript. AK, TM, SK and MT provided samples. TWJH and REMT helped with interpretation of the results. DvdW supervised project. All authors provided critical feedback.

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Ethical approval information, institution(s) and number(s)
The protocol for the Nagasaki Island Study was approved by the Ethics Committee for the Use of Humans of Nagasaki University (14051404). The EAC was approved by the medical ethical committee ('Commisie Medische Ethiek') of the Leiden University Medical Centre (LUMC) (B19.008). Written informed consent was obtained from all participants.

Data sharing statement
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request. Restrictions in the form of a data sharing agreement may apply.

Patient and Public Involvement
Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research

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