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 characterised 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

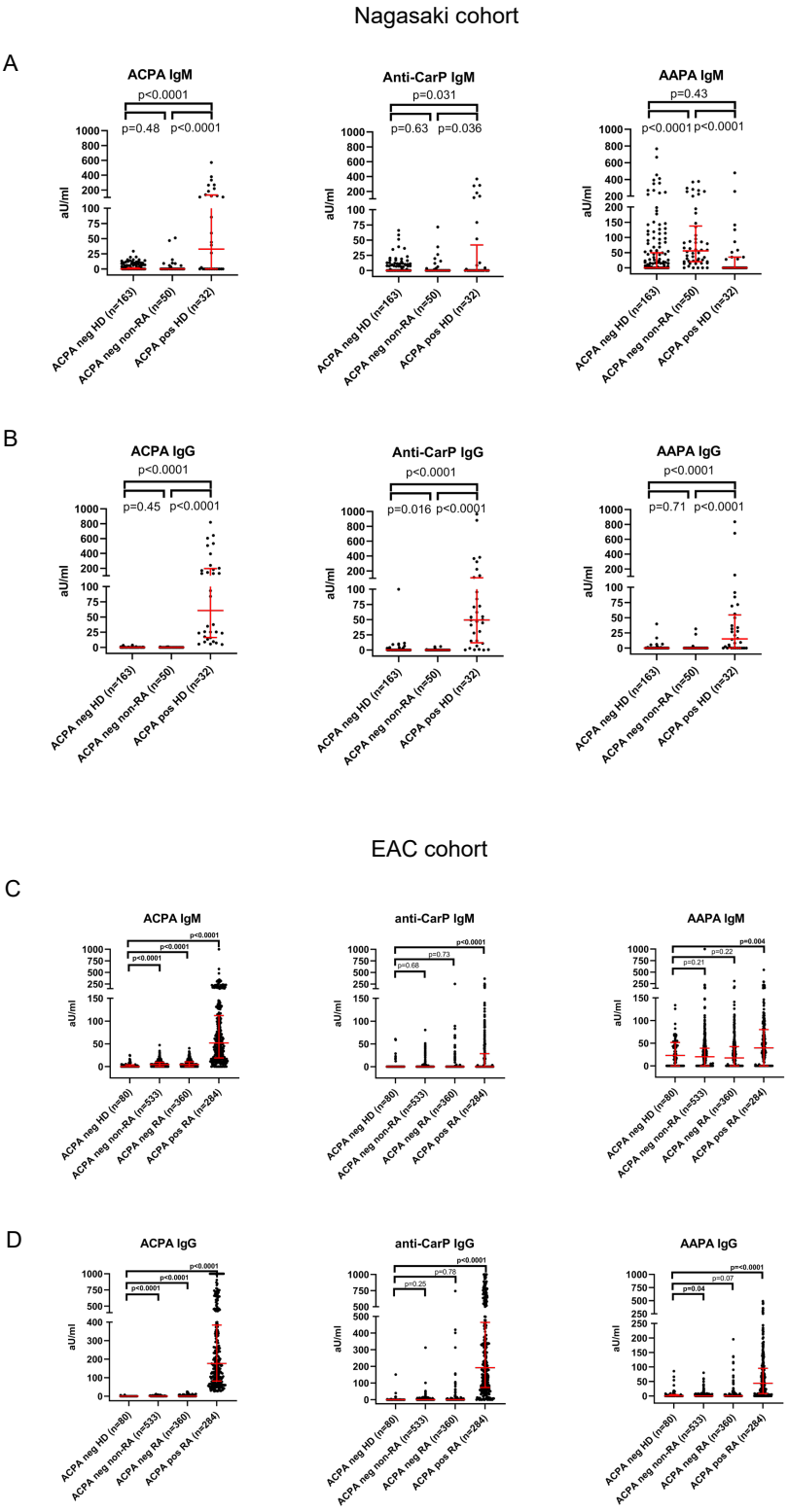

Figure 1 (A) ACPA-IgM, anti-CarP-IgM and AAPA-IgM levels in arbitrary units (aU) of Nagasaki Island cohort. (B) ACPA-IgG, anti-CarP-IgG and AAPA-IgG levels in aU of Nagasaki Island cohort. CCP4, CHcitP4 and CAcetylP4 were used as antigens. Medians with IQR are indicated in red. Signals from the control peptides CArgP4 and CLysP4 were subtracted from the CCP4 and CHCitP4 or CAcetylP4 signal, respectively. (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 IQR are indicated in red. Signals from the control peptide CArgP2, CLysP2 and CNorleuP2 were subtracted from the CCP2, CHcitP2 and CAcetylP2 signal, respectively. AAPA, anti-acetylated protein antibodies; ACPA, anti-citrullinated protein antibodies; anti-CarP, anti-carbamylated protein antibodies; CAcetylP2, anti-cyclic acetylated peptide 2; CAcetylP4, anti-cyclic acetylated peptide 4; CArgP2, anti-cyclic arginine peptide 2; CArgP4, anti-cyclic arginine peptide 4; CCP2, anti-cyclic citrullinated peptide 2; CCP4, anti-cyclic citrullinated peptide 4; CHcitP2, anti-cyclic homocitrullinated peptide 2; CHcitP4, anti-cyclic homocitrullinated peptide 4; CLysP2, anti-cyclic lysine peptide 2; CLysP4, anti-cyclic lysine peptide 4; CNorleuP2, anti-cyclic norleucine peptide 2; EAC, early arthritis clinic.

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 online supplemental material).

First, levels were determined in sera from the Nagasaki Island study[3] (online supplemental 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 (EAC) cohort,[4] showing high levels of ACPA-IgM and anti-CarP-IgM almost exclusively in patients with ACPA-IgG-positive RA, but not in HD (figure 1C and online supplemental table S4). Again, high AAPA-IgM levels were not only observed in patients with ACPA-IgG-positive RA, 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 and D and online supplemental 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 (online supplemental 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 (online supplemental 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 patients with ACPA-IgG-positive RA, although a few healthy individuals also have detectable AAPA-IgA levels (online supplemental figure S2A). Moreover, the correlation between AAPA-IgA and AAPA-IgG was stronger than the correlation between AAPA-IgA and AAPA-IgM (online supplemental 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 a non-specific binding (online supplemental figure S3). Inhibition experiments using different antigenic backbones provided further validation of the specificity of this response (online supplemental 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 (online supplemental 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 with 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-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 patients with arthritis 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.

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Correction notice This article has been corrected since it published Online First. The sentence 'The lack of reactivity to control peptides without PTMs confirmed that the AAPA-IgM signal was not due to non-specific binding.' has been corrected and the author equal contribution statement added.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and 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. Participants gave informed consent to participate in the study before taking part.

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REFERENCES

1 Juarez M, Bang H, Hammar F, *et al*. Identification of novel Antiacetylated Vimentin antibodies in patients with early inflammatory arthritis. *Ann Rheum Dis* 2016;75:1099–107.
2 Nielen MMJ, van Schaardenburg D, Reesink HW, *et al*. Specific Autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380–6.
3 Kawashiri S-Y, Tsuji Y, Tamai M, *et al*. Effects of cigarette smoking and human T-cell leukaemia virus type 1 infection on anti-Citrullinated peptide antibody production in Japanese community-dwelling adults: the Nagasaki Islands study. *Scand J Rheumatol* 2021;50:295–8.
4 van Aken J, van Bilsen JH, Allaart CF, *et al*. The Leiden early arthritis clinic. *Clin Exp Rheumatol* 2003;21(5 Suppl 31):S100–5.
5 Reijm S, Kissel T, Stoeken-Rijsbergen G, *et al*. Cross-reactivity of Igm anti-modified protein antibodies in rheumatoid arthritis despite limited mutational load. *Arthritis Res Ther* 2021;23.
6 Grönwall C, Liljefors L, Bang H, *et al*. A comprehensive evaluation of the relationship between different IgG and IgA anti-modified protein Autoantibodies in rheumatoid arthritis. *Front Immunol* 2021;12:627986.