Secretory form of rheumatoid arthritis-associated autoantibodies in serum are mainly of the IgM isotype, suggesting a continuous reactivation of autoantibody responses at mucosal surfaces

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The secretory form of rheumatoid arthritis associated auto-antibodies in serum are mainly of the IgM isotype, suggesting a continuous reactivation of auto-antibody responses at mucosal surfaces.

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Several lines of evidence obtained in recent years indicate a role of mucosal surfaces in the development of auto-immune responses associated with rheumatoid arthritis (RA). Therefore, more attention is going to the influence of the microbiome in RA development. Secretory-antibodies are typically produced at mucosal surfaces and transported through epithelial cells for secretion at the luminal site. Importantly, secretory-antibodies comprise both immunoglobulin-M (IgM) and immunoglobulin-A (IgA) and both isotypes can harbour a J-chain that binds to the polymeric Ig receptor (pIgR) [1]. Following transport through epithelial cells, the antibodies are enzymatically released at the luminal site by cleavage of the pIgR leaving a fragment of the receptor, the secretory-component (SC) bound to the immunoglobulin. SC-containing antibodies can however also be detected systemically in the circulation, although the mechanism by which these SC-containing antibodies arise in serum is still unclear [2]. Interestingly, antigen-specific secretory-antibodies are present in serum after mucosal immunization [3], indicating that the presence of secretory-antibodies may provide information about their origin; the mucosa. Well known auto-antibodies in RA are rheumatoid factor (RF), ACPA (against citrullinated proteins) and anti-CarP (against carbamylated proteins) [4]. Secretory-RF (SC-RF) and secretory-ACPA (SC-ACPA) have been detected in RA-sera [5, 6], indicating a mucosal origin. Although it is often postulated that SC-containing auto-antibodies are of the IgA isotype, this is unknown as both IgM and IgA can harbour SC. Nonetheless, it is important to define the isotype of SC-containing auto-antibodies as this provides relevant information on the type of B-cell (re)activated at mucosal sites.

Therefore, we determined whether secretory-anti-CarP (SC-anti-CarP), SC-ACPA and SC-RF are present in RA-patients and identified the isotypes used.SC-anti-CarP, SC-ACPA, SC-RF, SC-total-IgA and SC-total-IgM as well as total-IgA and -IgM were measured by ELISA in 363 RA-patients of the Leiden Early Arthritis Cohort (EAC) and 207 Healthy Controls (HC) [7, 8]. Groups were compared using non-parametric tests. Sera of 9 RA-patients were depleted for IgM or IgA after which SC-anti-CarP, SC-ACPA and SC-RF were determined.

The prevalence and levels of SC-anti-CarP, SC-ACPA and SC-RF are increased in RA-patients

compared to HC (prevalence: p<0.0001, levels: p<0.0001) (fig. 1a-c). Levels of SC-total-IgA and SC-total-IgM as well as total-IgA and total-IgM were increased in RA-patients compared to HC (p<0.0001 for all) (fig.1d,e,g,h). The increase of SC-total-IgM appeared RA-specific as no increase was observed in disease controls (fig.S1). Intriguingly and unexpectedly, we observed that the SC-auto-antibodies were predominantly present as IgM and not of the IgA isotype as mostly assumed (fig. 2). Indeed, the presence of SC-containing auto-antibodies correlates best with SC-total-IgM (fig.S2), also pointing towards their presence in the IgM isotype. The increased representation of SC-IgM auto-antibodies is not a general feature of SC-immunoglobulins present in RA, but likely more specific for auto-antibodies as correction for total levels of IgM normalized the observed increase in SC-total-IgM (fig. 1f,i). Importantly, the avidity of anti-CarP IgA and IgM, ACPA IgA and IgM and RF IgA and IgM are similar (fig.S3), indicating that the observed differences are not explained by "technical issues" related to the sensitivity of the methods used to detect SC-IgA vs SC-IgM auto-antibodies. Overall, SC-anti-CarP, SC-ACPA and SC-RF are present in- and specific for RA and consist predominantly of the IgM isotype. These findings are of importance as this indicate that especially autoreactive IgM-expressing B-cells represent the most prominent B-cell subset that is reactivated at mucosal areas, possibly (re)activating and keeping the immune response ongoing. This (re)activation could involve both naïve and pre-existing memory IgM B-cells or even (gut) IgM+ plasma cells directed against commensals [9] , possibly pointing to a role of the microbiome in steering RA-specific auto-immune responses.

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Competing interest

REMT and LAT are listed as inventors in a patent application regarding the detection of anti-CarP antibodies for RA.

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Figure legends

Figure 1. Secretory anti-CarP, ACPA and RF are increased in rheumatoid arthritis patients.
ELISAs were performed to detect secretory anti-CarP antibodies (A), secretory ACPA (B) and secretory RF (C) in sera of 207 HC and 363 RA-patients. Next to this total secretory IgA (D), total-IgA (E), AU secretory IgA per mg total-IgA (F), total secretory IgM (G), total-IgM (H) and AU secretory IgM per mg total-IgM (I) were measured in all HC and RA-patients. Plates were coated with Ca-FCS (A), CCP2 (B) and human IgG (C) and after serum incubation bound antibodies were detected using goat-anti-human secretory component (Nordic Mubio). For the detection of total secretory IgA and IgM, plates were coated with mouse-anti-human secretory component (SPM217) and after serum incubation bound antibodies were detected using goat-anti-human IgM (Millipore) or IgA (Novex). Total-IgA and -IgM was measured using the Bethyl kit (manufactures protocol). The 97th percentile in HC was used as cut-off for the presence of secretory auto-antibodies. The dotted line represents the cut-off and the red line the median. The green lines represent the "normal" range for total-IgA and -IgM in serum. The specific secretory antibody reactivity is depicted in arbitrary units per millilitre and total-IgA and -IgM in milligram per millilitre. Please note, as levels are depicted in AU, no direct comparisons in absolute antibody-levels can be made. The number of samples tested and the percentage positivity is shown below the graphs. Mann-Whitney U tests were carried out to determine differences in antibody levels between RA-patients and HC. The Pearson chi-squared test was used to determine differences in positivity between RA-patients and HC. HC; healthy controls, RA; rheumatoid arthritis, anti-CarP antibody; anti-carbamylated protein antibody, ACPA; anti-citrullinated protein antibodies, RF; rheumatoid factor, AU/ml; arbitrary units per millilitre, mg/ml; milligram per millilitre. Mann-Whitney U test *p=0.05-0.002, **p=0.002-0.0002, *** p=0.0002-0.0001, **** p< 0.0001

Figure 2. Secretory anti-CarP, ACPA and RF are predominantly of the IgM isotype.
Serum samples of 9 RA-patients were depleted for IgM (A-F) or IgA (G-L) and the presence of secretory anti-CarP antibodies, secretory ACPA and secretory RF (D-F, J-L) as well as total-IgM, -IgA and -IgG (A-C, G-I) was analysed in the start and depleted material. Plates were coated with CaFCS (D,J), CCP2 (E,K) and human IgG (F,L) and after serum incubation bound antibodies were detected using goat-anti-human secretory component (Nordic Mubio). Total-IgM, -IgA and -IgG was measured using the Bethyl kit (manufactures protocol). Every colour represents a patient. anti-CarP antibody; anti-carbamylated protein antibody, ACPA; anti-citrullinated protein antibodies, RF; rheumatoid factor, AU/ml; arbitrary units per millilitre, mg/ml; milligram per millilitre.

Supplementary figure 1. Secretory auto-antibodies have the strongest correlation with secretory IgM. To investigate correlations between secretory auto-antibodies and secretory IgA (A,C,E) or secretory IgM (B,D,E) Spearman Rank tests were carried out on all tested RA-patients. anti-CarP antibody; anti-carbamylated protein antibody, ACPA; anti-citrullinated protein antibody, RF;rheumatoid factor

Supplementary figure 2. The anti-CarP, ACPA and RF IgA and IgM avidity is similar.
Elution ELISA assays were performed to test the anti-CarP antibody, ACPA and RF IgA and IgM avidity on Ca-FCS, CCP2 and human IgG in sera of 4 RA-patients. The avidity index (AI) is depicted as % restbinding at 1M NaSCN. anti-CarP antibody; anti-carbamylated protein antibody, ACPA; anti-citrullinated protein antibody, RF;rheumatoid factor, Ca-FCS; carbamylated fetal calf serum, CCP; cyclic citrullinated peptide, RA; rheumatoid arthritis, AI; avidity index , NaSCN; sodiumthiocynate

Figure 1 M.A.M. van Delft

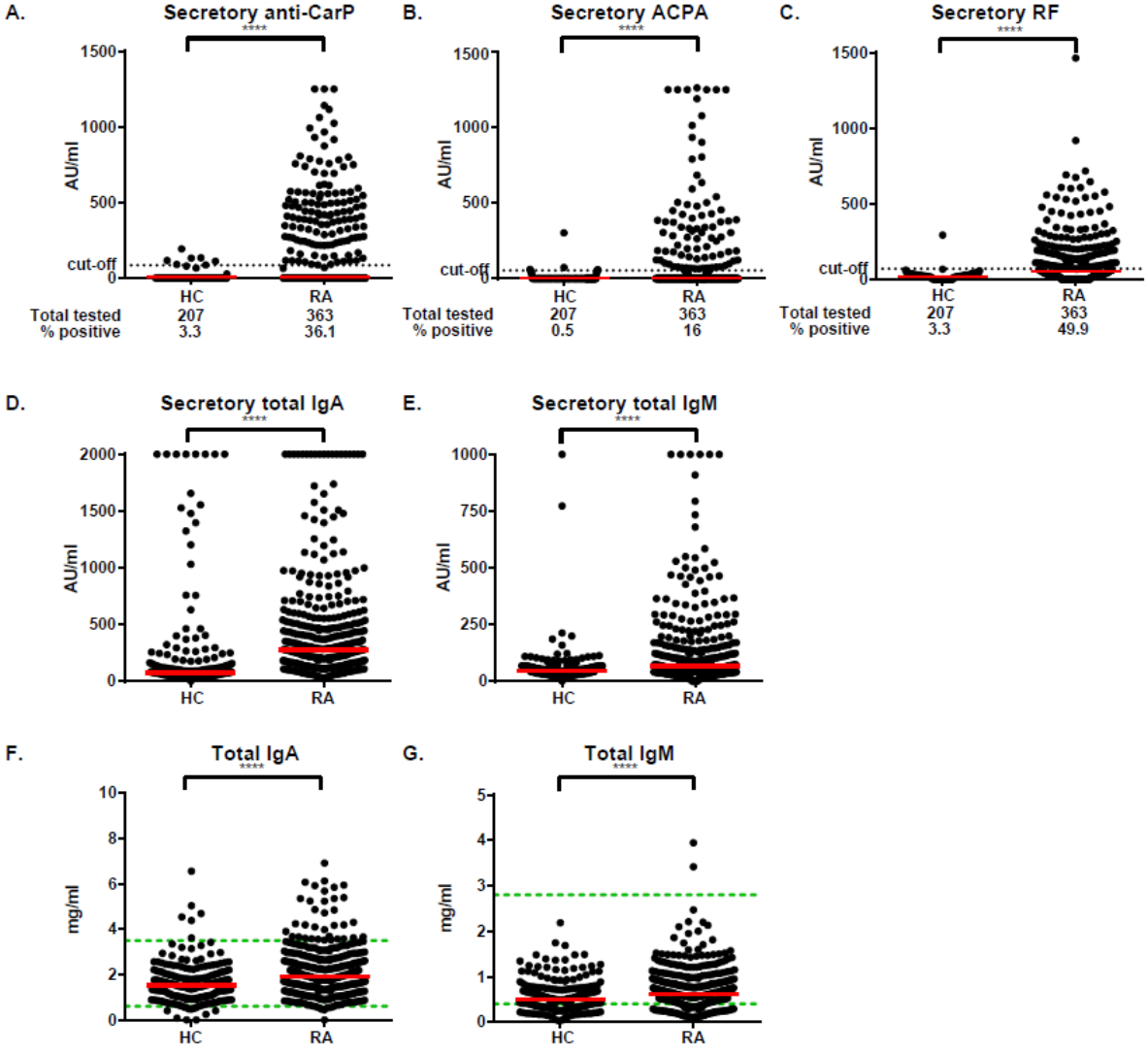
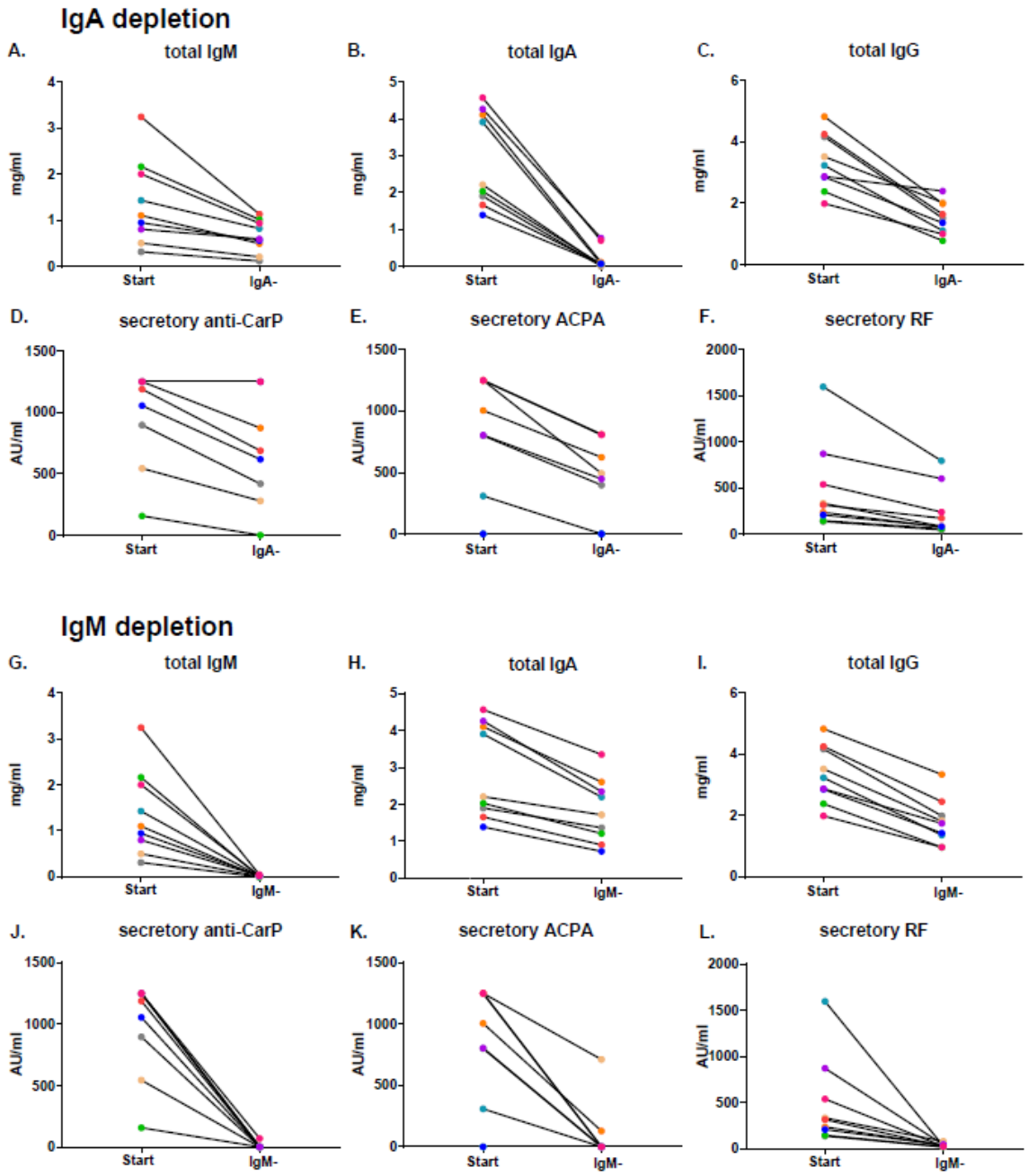
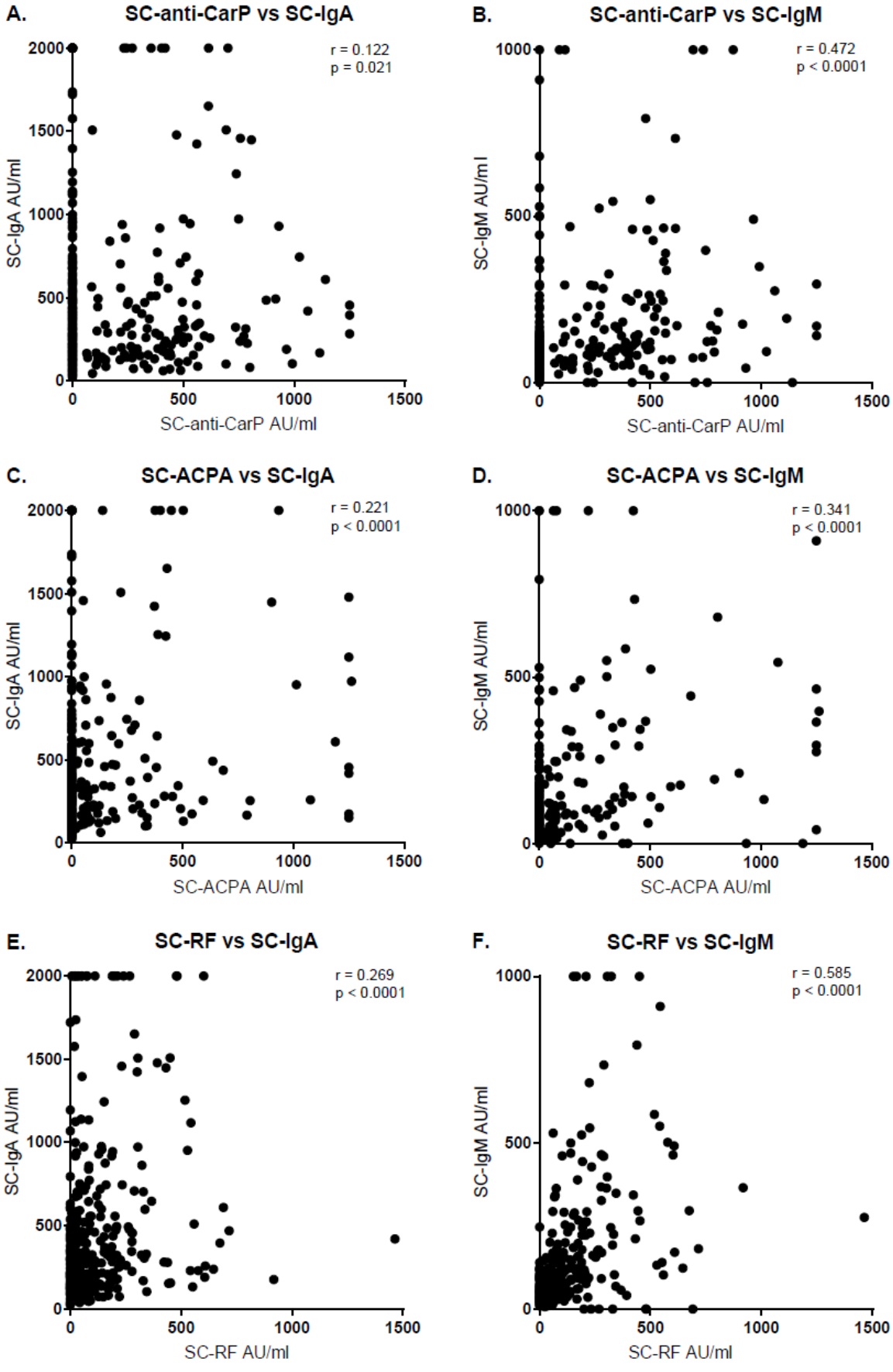


Figure 2


IgA depletion
A.
total IgM
mg/ml
Start
IgA-
B.
total IgA
C.
total IgG
D.
secretory anti-CarP
AU/ml
E.
secretory ACPA
F.
secretory RF
IgM depletion
G.
IgM-
H.
I.
J.
K.
L.

Supplementary Figure 1



Supplementary Figure 2