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Acquiring new N-glycosylation sites in variable regions of immunoglobulin genes by somatic hypermutation is a common feature of autoimmune diseases

With great interest, we read the contribution of Vergroesen *et al* that was published recently in *Annals of the Rheumatic Diseases*.¹ In this manuscript, the authors describe the observation that immunoglobulin variable (V) region heavy and light chain transcripts from anti-citrullinated protein antibody (ACPA) IgG-expressing B cells in patients with rheumatic arthritis (RA) contain N-glycosylation sites (Nglycs) acquired by somatic hypermutation, whereas these acquired Nglycs (ac-Nglycs) were absent in tetanus toxoid (TT) specific B cells of healthy individuals. The authors postulate that the introduction of ac-Nglycs generates selective advantages that allow ACPA-expressing B cells to escape from classical selection mechanisms in germinal centres. We agree with the authors that this is an important finding which may have important implications for understanding citrulline-specific immunity in RA.

Here we would like to stress that ac-Nglycs, as a consequence of somatic hypermutation, might be important for RA and for many other rheumatoid and non-rheumatoid autoimmune diseases. We have shown previously² increased numbers of IgG encoding immunoglobulin variable heavy region gene (IGHV) transcripts with ac-Nglycs derived from B cells and plasma cells residing in the inflamed parotid salivary gland of patients with primary Sjögren's syndrome (pSS) compared with non-pSS sicca controls (24% vs 6%). Importantly, in pSS the IgG encoding transcripts exhibited no evidence for signs of antigen selection in the complementarity determining regions. Furthermore, most (60%) of the ac-Nglycs were seen in the framework (FR) 3 region, which is similar to the findings in ACPA-expressing B cells.¹ Together, these findings indicate that alternative selection mechanism may result in survival of aberrantly selected B cells in autoimmune diseases, like pSS and RA.

To test if ac-Nglycs created by somatic hypermutation could be a common phenomenon for B cells in rheumatic and non-rheumatic autoimmune diseases, we performed a meta-analysis of the presence of ac-Nglycs in IGHV sequences from a number of publicly available data sets from patients with pSS (n=576), RA (n=1331), systemic lupus erythematosus (SLE; n=361), multiple sclerosis (n=200), chronic Chagas' heart disease (cChD; n=70), ankylosing spondylitis (n=29) and granulomatosis with polyangiitis (GPA; n=242). As controls, we collected published data sets of IGHV sequences derived from various healthy, non-autoimmune individuals (n=2131) and from vaccination or infection studies on antigen-specific B cells (n=817) (see online supplementary table 1). Sequences identical to germline IGHV sequences (≤ 2 nucleotide mutations in the V and J genes), naturally occurring germline Nglycs and sequences lacking fully designated VDJ rearrangements were excluded from analysis. In each study, the number of ac-Nglycs was predicted by the NetNGlyc V1.0 program (<http://www.cbs.dtu.dk/services/NetNGlyc/>) based on the consensus sequence in the protein motif N-X-S/T (asparagine-X-serine/threonine). As shown in figure 1A, the combined frequency of ac-Nglycs in IGHV sequences is significantly higher ($P < 0.0001$; Pearson's χ^2 test) in autoimmune disease data sets (9.0%; 95% CI 8.0 to 10.1) than that in control data sets (2.3%; 95% CI 1.7 to 3.0) as well as in antigen-specific data sets (2.7%; 95% CI 1.7 to 4.0).

Interestingly, the number of ac-Nglycs is elevated in nearly all autoimmune diseases with well-established B cell involvement except for GPA (figure 1B); we observed a higher amount of ac-Nglycs in the IGHV sequences from pSS (15%), RA (10%), SLE (6%), multiple sclerosis (9%) and for cChD (19%) compared with the normal control data set (3%). Although GPA is an autoimmune disease, well known for its B cell involvement, ac-Nglycs were completely absent in the IGHV sequences. Possible explanations could be the over-representation of IgM encoding sequences in these data sets (online supplementary table 1). In patients with ankylosing spondylitis, the number of ac-Nglycs (3%) is similar to normal controls.

Figure 1 Prevalence of acquired N-glycosylation sites in IGHV sequences. (A) This figure depicts a simplified version of the meta-analysis shown in online supplementary figure 1. The percentages of ac-Nglycs were compared between autoimmune diseases and non-autoimmune or normal controls (N) and vaccination and infection controls (AgS) regardless of isotype. All the studies combined revealed 275 ac-Nglycs out of 2809 IGHV sequences from patients with autoimmune disease (9.0%; 95% CI 8.0 to 10.1) which was significantly higher than the 64 ac-Nglycs out of 2131 IGHV sequences in the healthy controls (2.3%; 95% CI 1.7 to 3.0) and the 23 ac-Nglycs out of 817 IGHV sequences in the vaccination and infection controls (2.7%; 95% CI 1.7 to 4.0). Because of the different sizes of all the data sets, analysis of the data was performed by using the Freeman-Tukey arcsine transformation method for meta-analysis of proportions to estimate the pooled proportion (read combined frequency) with the 95% CIs for each data set (see also online supplementary figure 1). The P values were calculated with the Pearson's χ^2 test. (B) Distribution of the frequency of ac-Nglycs in the IGHV sequences in the different autoimmune diseases. The percentage of ac-Nglycs found in the IGHV sequences from the data sets was calculated for each autoimmune disease. The number of observed ac-Nglycs per total number of analysed IGHV sequences is indicated in each bar. AS, ankylosing spondylitis; cChD, chronic Chagas' heart disease; GPA, granulomatosis with polyangiitis; MS, multiple sclerosis; pSS, primary Sjögren's syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

Correspondence

In conclusion, in addition to Vergroesen *et al* in RA, and our previous work in pSS,² the meta-analysis described here clearly indicates that there is an increase in ac-Nglycs by somatic hypermutation of immunoglobulin genes during humoral immune responses in various autoimmune diseases. This phenomenon is thus clearly not restricted to ACPA-expressing B cells in RA as shown by Vergroesen *et al*. It is not known yet whether the absence of ac-Nglycs in TT-specific cells is a property of TT specificity or due to the fact that these cells are from healthy, vaccinated individuals. It would therefore be of great interest to see whether also in patients with RA ac-Nglycs are absent in TT-specific B cells and other non-ACPA-expressing B cells. The explicit tendency for ac-Nglycs to occur also within the FRs strongly suggests that the increased frequency of ac-Nglycs in autoimmune diseases may offer Ig-producing cells alternative forms of selection to classical antigen selection. This could point to a very fundamental basis to understand the origin of autoreactive B cells in autoimmune diseases and possible targets for early intervention.

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Contributors AV wrote the concept of manuscript and performed the analysis of the data sets. NH performed the initial analysis of the data set. NAB and FGMK were involved in writing and discussion of the data.

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