ANTI-MODIFIED PROTEIN AUTOANTIBODIES IN RA
DISPLAY IMPORTANT PEPTIDE CROSS-REACTIVITY
BUT YET PROTEIN RECOGNITION SELECTIVITY

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Background: The continuing increase of anti-citrullinated protein autoantibodies (ACPAs) titers together with epitope spreading close to onset of disease, suggests that antibody responses to different citrullinated anti-gens may be critical in rheumatoid arthritis (RA) pathogenesis. Interestingly, monoclonal antibodies demonstrate reactivity to multiple cit-antigens that can even expand to other protein modifications. The width of this cross-reactivity is still not understood.

Objectives: To characterize the targets of monoclonal ACPA in relation to amino acid motif recognition, cross-reactivity with others post-translational modifications, and cellular localization.

Methods: A peptide array ( NimbleGen, Roche) containing 16aa arginine- or lysine in pairs with citrulline- or homocitrulline peptides (53019 and 49211 cognate peptide pairs, respectively) derived from 1610 extracellular proteins and known RA cit-targets was used to screen 12 monoclonal ACPA with CCP2 reactivity. In addition, these ACPAs were also screened for reactivity to acetylated-histone peptides and for reactivity to acetylated Hela cell extracts from cytosol, membrane, nuclear and cytoskeleton fractions. Three of the described mAbs together with polyclonal anti-CCP2 Gg were further evaluated on a macroarray platform (HEXselect, Engine) consisting of 20776 E.coli on-array expressed His-tagged protein fragments from 6909 genes originating from a human cDNA library. The array was enzymatically citrullinated with rabbit PAD and mAb-reactivity was scored from 0-3.

Results: On the peptide arrays, all 12 ACPA displayed low reactivity to unmodified peptides (<0.06%), while reacting to 1,000s of synthetically citrullinated peptides (>3.4% of the peptides). Based on the sequence from the positive peptides, consensus amino acids motifs were created, identifying as-patterns with only a few critical citrulline-flanking residues (e.g. Cit-Gly: Oly-Cit; Arg-Cit-Asp). Intriguingly, 5 of the antibodies also reacted with the carboxamidated peptides (>2.2%) and the recognition of certain homocitrulline-motifs also correlated with cross-reactivity to acetylated peptides. Interestingly, these AMPA reacted with acetylated-histones in NETs and apoptotic cells and in the nuclear fraction of in vitro acetylated cell-extracts. Three of the 12 ACPA were further screened on the macroarray and displayed multiple binding to citrullinated proteins and protein fragments identifying primarily previously unknown autoantibody targets (96, 210 or 917 positive hits for the mAbs, scoring 2-3), while limited binding was seen to native proteins.

Conclusion: ACPA display multi-reactivity to citrullinated peptides and proteins to a much greater extent than previously appreciated. Additionally, some ACPA, but not all show distinct cross-reactivity to other post-translational modifications. Importantly, different autoimmune clones display modified protein recognition patterns dominated by proteins from different cellular structures. These reactivity profiles are likely to have impact on functionality and pathogenesis.


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SUPPORTING EARLY CAREER RESEARCHERS IN RHEUMATOLOGY AND MUSCULOSKELETAL MEDICINE: RESULTS FROM AN EMERGING EULAR MEDICINE (EMEUNET) SURVEY

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Background: Early career researchers (ECRs) across Europe face a number of challenges as highlighted by the European federation of education employers (EFEE) (1). Understanding the unmet needs of ECRs in rheumatology would allow development of targeted educational resources and support where required.

Objectives: To perform a Europe wide survey on the demographics of ECRs, current unmet needs and perceptions of possible solutions.

Methods: Clinical and non-clinical researchers who work in the field of rheumatology and under the age of 40, were invited to participate in the online-based survey. EMEUNET is a Europe-wide network of >2000 young researchers in rheumatology addressing educational needs and promoting research interests. Survey questions were devised and modified in collaboration with members of the EMEUNET education subgroup and steering committee. It was disseminated to EMEUNET members and national young rheumatology organisations. Participants were allowed to choose more than one answer option and the survey was applicable for individuals aged 18 years or older.

Results: 339 participants’ anonymised responses were collected from 53 countries. The majority of participants were between 31-35 years (38.1%) and female (63.4%). Most were clinical researchers (including 33.3% rheumatology trainees) and 24.1% were non-clinical, including allied health professionals. The area of research of the participant was as follows: Epidemiology (40.0%), basic science/translational (39.6%), clinical trials (38.1%), imaging (17.7%), health services research (14.7%), other (7.5%). 48.6% did not feel they had adequate educational resources to develop their research skills locally. Obtaining grant funding as ECRs was deemed to be difficult (43.3%) or very difficult (31.4%) in their respective institutions/countries. Reasons listed are presented in figure 1. 98% were interested in developing new European collaborations in their research area either through: face to face interactions at conferences (82.1%), website forum (50.8%), email interactions (61.1%), teleconferences (43.5%). In addition, 93.7% felt they would apply for small European grants for ECRs, 81.6% would be interested in funding to spend short periods (4-8 weeks) at another European institute and 87.5% in focussed deep dive sessions on a topic of interest. The top 3 research skills that participants felt they would benefit from having more resources for as ECRs were: 1. Writing a study protocol (64.9%), 2. Writing a first grant application (64.0%), 3. Performing a systematic review (51.1%) (Figure 2).

Conclusion: A large proportion of ECRs in rheumatology felt they lack resources to develop their research skills locally. Small grant funding, research opportunities for pan-European collaborations, short periods of exchange to other institutions and targeted support to develop research skills can help address some of the current needs of ECRs.