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David Hutchinson MD[1] [2], Daniel Murphy BmBs[1], Alex Clarke BSc[2] and Paul Eggleton MPhil, PhD[2]

1 Department of Rheumatology, Royal Cornwall Hospital Trust, Truro, Cornwall TR1 3LQ

2 University of Exeter Medical School, Exeter, Devon EX1 2LU

Competing interests

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Correspondence to:

Dr. Daniel Murphy,

Department of Rheumatology, Royal Cornwall Hospital Trust, Truro, Cornwall TR1 3LQ.

E-mail: Daniel.Murphy8@nhs.net

Phone: 01872 250000

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To the editor. We read with interest the recent article by Koppejan et al [1] where a strong assocation between rheumatoid arthritis (RA) and triple positivity for rheumatoid factor (RF), anti-citrullinated protein antibodies (anti-CCP) and anti-carbamylated protein antibodies (anti-CarP) (odds ratio (OR) 194 95% CI (23-1609), p<0.0001) was observed. The authors suggest that these findings offer new insights into the evolution of autoimmunity in preclinical RA.

We suggest that misfolding of immunoglobulin G (IgG) in the lung has the potential to initiate seropositive RA, sequentially generating the three RA autoantibodies observed in the above study. We propose a pathway relevant to smokers and individuals carrying the HLA class II shared epitope (SE), explaining the very high prevalence of smoking and the SE noted in RA patients [1]. Immunoglobulin G is a plausible “universal” antigen for all three known RA autoantibodies, as although there are a plethora of proteins that can act as antigens to both anti-CCP and anti-CarP antibodies, RF only has one antigen.

Rheumatoid factor stage: smoking-induced bronchial associated lymphoid tissue (BALT) and an increased generation of IgG

Smoking associates with the development of BALT, a process associated with seropositive RA [2]. This plasma cell abundance potentiates increased IgG production, evidenced by OR for the development of RF-positive RA in the lowest compared to the highest tertiles of IgG distribution in individuals prior to disease development being 2.22 (95% CI 1.16, 4.26) [3]. IgA RF is present in the sera of individuals who subsequently develop seropositive RA up to 15 years before disease development and is observed

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prior to both ACPA and anti-CarP [4]. We propose that this initial autoantibody response may represent a signal of increased lung IgG production, with IgA RF antibodies responding to native IgG protein.

Citrullinated IgG heavy chain (cIgGH) generation stage: misfolding induced by citrullination

Protein misfolding is enhanced by citrullination [5] and IgG misfolding has been suggested as a model of RA autoimmunity with the generation of IgGH, a 50 kilodalton (kDa) post-translationally modified IgG heavy chain fragment [6]. Growth arrest and DNA damage-inducible gene 34 has an important role in the unfolded protein response and significantly relates to the presence of ACPA in RA [7], suggesting ACPA generation associates with protein misfolding. Given that the lung is a site of citrullination [8], we suggest that the intracellular citrullination of IgGH occurs in lung plasma cells resulting in the development of the novel neoantigen cIgGH.

Anti-citrullinated protein antibody stage: shared epitope dependant pathway of cIgGH cellular escape and extra-cellular antigenicity

Under normal circumstances misfolded proteins are processed to peptides. Interestingly, it has been suggested the transport of IgGH out of cells occurs as a complex with SE alleles as this specific complex is recognised by autoantibodies occurring in RF-positive RA patient's sera, but not by autoantibodies in RF-positive sera from individuals without RA [6]. We suggest further that the process of IgGH-SE binding is greatly enhanced by citrullination, given that citrullination of calreticulin enhances SE

binding by 10000-fold compared to the native protein [9]. Once the cIgGH is extracellular, specific ACPA are likely to develop, with cross reactivity giving rise to a positive anti-CCP result.

Anti-carbamylated protein antibody stage: smoking induced carbamylation of cIgGH

Cigarette smoking associates with raised serum levels of thiocyanate and myeloperoxidase-catalyzed oxidation of thiocyanate results in increased carbamylation in smokers [10]. Recently, vimentin carbamylation was noted in chronic obstructive pulmonary disease lung samples [8]. Ergo, we suggest that IgGH is also carbamylated in the lung. Importantly the carbamylation of cIgGH may greatly diminish the clearance of this antigen locally by the classical complement pathway as carbamylation of IgG abrogates activation of the classical complement pathway [11]. This process may give rise to large quantities of uncleared carbamylated cIgGH entering the circulation and generation of a positive anti-CarP response.

RA initiation stage: Circulation of carbamylated cIgGH

The synovial fluid of RA patients has been observed to contain specific IgG which has the potential to stimulate an intense antibody formation when injected into mice which is not observed with native IgG [12]. This response was characterized by high and sustained levels of IgG1 antibodies with RF activity. We propose that the generation in the lung of carbamylated cIgGH leads to circulation of this antigen with the potential to enter the joint and suggest that carbamylated cIgGH initiates a cascade of B-cell

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activation, with the development of tertiary lymphoid tissue, further IgG misfolding and RA development, explaining the authors observation that when all three autoantibodies are present there is a strong association with RA.

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