Review

The lung in ACPA-positive rheumatoid arthritis: an initiating site of injury?

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Abstract

Recent findings have highlighted the potential initiation of ACPA in sites away from the joint. Periodontitis is an example of this concept. This process in the gums appears to be independent of smoking, the main environmental risk factor for ACPA-positive RA. There is extensive literature regarding the potential role of smoking in the pathogenesis of ACPA-positive RA. As a consequence of this strong association, the lung has become the focus of research to determine whether processes within the lung are linked to the generation of ACPA. Here we outline the current body of evidence and explore the hypothesis that the lung as an organ of immune defence has a role in the pathogenesis of the autoimmune disease ACPA-positive RA.

Key words: anti-citrullinated protein/peptide antibodies, rheumatoid factor, autoimmunity, infection, interstitial lung disease, bronchiectasis, cigarette smoking, rheumatoid arthritis, pathogenesis.

Introduction

RA is a heterogeneous disease. The subdivision of RA by autoantibody status, traditionally RF and more recently ACPA, has facilitated greater understanding of potential pathogenic mechanisms in the development of RA. This is especially the case for ACPA-positive RA, where RA initiating sites distant from the joints have been proposed within the gastrointestinal and respiratory systems.

Within the gastrointestinal tract the bacterium \textit{Porphyromonas gingivalis} is linked to the gum disease periodontitis [1]. Periodontitis increases the risk of RA in non-smokers and is associated with ACPA production [2]. A recent study demonstrated that \textit{P. gingivalis} facilitated the development and progression of ACPA-positive RA through its unique bacterial peptidylarginine deaminase (PAD) [3]. The link between periodontitis and ACPA-positive RA emphasizes that processes outside the synovium are important in the pathogenesis of ACPA-positive RA.

Within the respiratory system, cigarette smoke is a major environmental risk factor for RA [4, 5]. Increased citrullinated peptides are found in the lung tissue of smokers [6, 7] and evidence suggests that smoking in the presence of HLA-DRB1-shared epitope alleles may initiate immunity to citrullinated peptides and lead to the development of ACPA-positive RA [8]. In addition to the increase in ACPA prevalence in smokers, [9] increased ACPA prevalence is recognized in RA-related lung diseases [10], including patients with RA and bronchiectasis (RA-BR), a condition with a high prevalence of never smokers [11, 12]. Infections also increase citrullination of peptides [13] and respiratory microorganisms are linked to the development of RA [14]. Here we highlight the recognized respiratory conditions associated with RA, their RA autoantibody associations and the potential role for RA-related lung disease in citrulline immunity and the subsequent development of ACPA-positive RA.

Immunity in the respiratory system

A fine balance exists between immune activation to eradicate pathogens and immune regulation to avoid excessive immune response that may impact on the physiological role of the respiratory system. However, with age the lung is vulnerable to increased inflammation from both infection and autoimmunity [15]. Immune responses are tightly controlled, particularly so within the low bacterial load environment of the terminal bronchioles and alveoli. Responses here are typically anti-inflammatory in nature to prevent impairment of gas exchange due to excessive...
Some mechanisms of innate and adaptive immunity involved in the antimicrobial defence of the respiratory system are summarized in Table 1.

**RA autoantibodies**

Autoantibodies are found against many host components in RA patients, but the RA autoantibodies RF and ACPA are the most widely used biomarkers in the diagnosis and prognosis of RA in clinical practice. RF and ACPAs are present in 70–80% of RA cases [16] and develop prior to the onset of clinically detectable RA [17, 18] or subclinical RA [19].

Traditionally RA is divided into subsets defined by the presence/absence of a certain titre of RF. RF is directed against the Fc component of IgG. Conventional assays detect IgM RF, but IgG and IgA RF can be detected using specialized assays. IgA RF in particular presents prior to clinically detectable RA and is thought to have a primary role in pathogenesis [18]. Although the exact mechanism is unknown, RF may drive the RA inflammatory process by formation of immune complexes that trigger complement activation and cytokine release by leucocytes [20]. The utility of RF as a clinical biomarker is limited by low specificity. More recently the presence/absence of specific ACPAs has proven to be a more informative biomarker in the subdivision of RA.

ACPAs are highly specific for RA (95–98% [21]) and represent a subset of RA that differs in terms of pathogenesis [7], disease course [22] and response to therapy [23, 24] when compared with ACPA-negative RA. Primary targets for ACPA identified by direct study of inflamed tissue and sera include a number of citrullinated connective tissue proteins/peptides, including vimentin (a type II filamentous protein) [25], fibrinogen [26], α-enolase [27] and collagen type II [28]. These antigens probably represent a fraction of the proteins recognized by ACPA in RA patients.

Studies comparing healthy relatives of ACPA-positive patients, individuals with undifferentiated arthritis (UA) and subjects with fully established RA demonstrate that the ACPA response evolves over time. In particular, ACPA isotypes expressed by asymptomatic ACPA-positive patients and patients with UA tend to be more restricted, often consisting primarily of IgA and IgG1 isotypes [29, 30]. IgM ACPAs can be indicative of an ongoing immune response and persist in UA patients whose disease evolves into RA and in patients with established RA [30].

The association between RF and ACPAs in patients with UA who develop RA and in patients with established RA suggest that although these autoantibodies seem to develop independently, they may play a synergistic role in disease initiation and progression by an unknown mechanism. However, murine models suggest that autoantibody-mediated articular inflammation is dependent on soluble immune complexes that can access the articular surface [31]. RF antibodies recognize IgG molecules, hence it is possible they form soluble immune complexes that facilitate access of ACPAs to the articular surface. An alternative proposed mechanism is that RF amplifies the effector mechanism of ACPA [29].

In the UK, ACPA-positive RA is diagnosed by the second-generation anti-CCP test, an ELISA developed using synthetic CCPs as antigens [32]. It is worth noting that synthetic CCPs are not true physiological proteins, therefore, although an excellent diagnostic tool for identifying reactivity to citrullinated proteins/peptides,
anti-CCP testing gives no detail on specific immune reactivities to the physiological citrullinated antigens present in the patient, as outlined above.

Genetic variability and adaptive immunity in ACPA-positive RA

Genetic variations in the MHC, class II, DRβ1 (HLA-DRB1) and protein tyrosine phosphatase (PTPN22) genes are major risk factors for the development of ACPA-positive RA [33]. Notably, both are thought to have functions in the adaptive immune system: HLA-DRB1 MHC molecules present antigens to T cells, whereas the protein tyrosine phosphatase coded for by PTPN22 appears to have an important function in the regulation of T cell activation [34].

The HLA-DRB1 alleles associated with RA encode a common five peptide sequence at amino acids 70-75 (DQRAA) in the third hypervariable region of the β-chain, referred to as the shared epitope (SE; HLA-DRB1 SE) [35, 36]. The frequency of different SE-containing alleles in RA populations varies by racial/ethnic group, as illustrated in Fig. 1, and comprise a major risk factor for erosive RA [37].

Importantly, citrullination of peptides shows increased affinity of their interaction with SE-containing MHC molecules [39]. This peptide–SE complex is then thought to present to CD4+ T cells, which activate B cells driving ACPA production [40]. Supporting this hypothesis, Bellatin et al. [40] showed that the production of IgG anti-CCP by B cells of RA patients in vitro is associated with carriage of HLA-DRB1 SE.

Kallberg et al. [33] demonstrated that the PTPN22 at risk allele R620W is only associated with ACPA-positive RA in the presence of the SE (HLA-DRB1 SE) and that the interaction between HLA-DRB1 SE, PTPN22 R620W and the environmental risk factor of smoking is additive (Fig. 2) [33]. In other words, having antigen-presenting cells with MHC class II molecules containing SE alleles (component cause 1) and T cells with dysfunctional down-regulation associated with the PTPN22 R620W allele (component cause 2) increases the risk of ACPA-positive RA more than the expected sum of each separate cause [33].

The anti-CCP test does not demonstrate which immune reactivities to citrullinated peptides are present. Mahdi et al. [27] investigated the role of citrullinated α-enolase peptide (CEP-1) as a disease-specific autoantigen in RA and found that the additive associations with HLA-DRB1 SE, PTPN22 and smoking detailed by Kallberg et al. [33] were confined to the subset of RA patients who carry anti-CEP1 antibodies (Fig. 2).

Citrullination, α-enolase, autoimmunity and environmental triggers

The multifaceted glycolytic enzyme α-enolase is a heat shock protein that operates as a receptor and activator
For plasminogen and is also an Myc binding protein [41]. It is up-regulated during cell differentiation, hypoxia and by pro-inflammatory stimuli. In its native form it has been described as an autoantigen in various infectious and autoimmune conditions [41]. Autoimmunity to the citrullinated form of α-enolase is just one of a group of primary immunodominant citrullinated peptides identified as potential autoantigens in RA patients. For example, citrullination is a normal physiological response in skin keratinization; however, there is evidence to suggest it is enhanced in the pathogenesis of ACPA-positive RA.

Citrullinated α-enolase is present in rheumatoid synovial fluid [43] and found in increased concentrations in the lungs of smokers [44]. There is a growing body of evidence that citrullinated α-enolase may be a prominent autoantigen in the pathogenesis of ACPA-positive RA. Citrullination is catalysed post-translationally by PADs that deaminate arginine residues. Human PADs are Ca²⁺-dependent enzymes that convert peptidylarginine into peptidylcitrulline [45]. Five main isoforms of PAD exist in different tissues: PAD1 and PAD3 are found predominantly in the epidermis, PAD2 is found in brain tissue and haematopoietic cells and PAD4 and PAD6 are found in haematopoietic cells [13]. Notably, PAD2 and PAD4 have been found in synovial tissue [46].

Increased expression of PAD2 and PAD4 compared with healthy controls have been identified in bronchial lavage [6] and in lung tissue of smokers [43, 47]. Moreover, smoking can promote non-specific citrullination in the lung, predisposing to ACPA development [48]. Periodontitis is another environmental risk factor recognized to promote citrullination in RA. P. gingivalis is the major causative infective agent in periodontitis and the only bacterium known to express PAD. Porphyromonas PAD (PPAD) citrullinates both bacterial and host peptides [49]. Indeed, ACPAs to the immunodominant peptide CEP-1 have been shown to react with the corresponding region of citrullinated P. gingivalis enolase [50], supporting a possible role for microbial mimicry in the pathogenesis of RA. Furthermore, Quirk et al. [51] demonstrated that autocitrullination of PPAD and an increased antibody response to this enzyme are specific to ACPA-positive RA.

Two key observations support the concept that citrulline immunity in RA is initiated outside of the synovium. First, inflammation of the synovium does not coincide with the occurrence of RA autoantibodies [19]. Second, although citrullinated peptides are recognized as part of normal physiological responses outside the synovium, their absence in healthy joints [6] suggests ACPAs develop outside the synovium and that a second hit is required, e.g. trauma/local inflammation within the synovium, to trigger citrullination of peptides [19] and drive the development of ACPA-positive RA (Fig. 3).
Bronchiectasis

Bronchiectasis is the anatomical distortion of conducting airways that results in chronic cough, sputum production and recurrent infection [60]. It has many causes and disease associations, however, 50% of cases are of unknown aetiology and termed idiopathic [61]. RA patients have an increased prevalence of symptomatic bronchiectasis compared with the general population [62]. The prevalence of anatomical changes consistent with bronchiectasis on HRCT studies is reported in up to 30% [53, 63], illustrating that many RA patients may have subclinical structural bronchiectasis.

We have demonstrated anti-CCP positivity in 94% and RF positivity in 97% of patients with RA-BR, which is significantly higher than would normally be seen in patients with RA alone [12]. The mechanism is unknown, although RA-BR patients are frequently never smokers [11], hence a group that we would not expect to be at high risk of ACPA-positive RA. An alternative trigger may drive the development of ACPA-positive RA in this group. Given the high prevalence of anatomical structural changes consistent with bronchiectasis on HRCT of RA patients, it is possible that this trigger is in the lung.

In addition, compared with ethnicity-matched control populations with RA alone, an association with HLA-DRB1*0405 has been identified in a cohort of Japanese RA-BR patients [64] and an association with the SE allele HLA-DRB1*0401 has been identified in a cohort of French RA-BR patients [65]. This strengthens the hypothesis that the presence of bronchiectasis with or without
recurrent infection may drive the development of RA autoantibodies in susceptible individuals (HLA-DRB1 SE carriers).

Interstitial lung disease
The term ILD embraces a group of inflammatory disorders and possibly pulmonary interstitial fibrosis whose progression results in impaired oxygen transfer and scarring of the lung [66]. Depending on the diagnostic criteria applied, in unselected populations HRCT changes consistent with ILD can be seen in up to two-thirds of RA patients [67, 68].

Usual interstitial pneumonia (UIP) is the predominant histological finding in RA-ILD, affecting more than half of patients [69]. Reticular markings and peripheral honeycombing on HRCT characterize UIP, whereas ground glass infiltrates typify non-specific interstitial pneumonitis (NSIP) (one-third of RA-ILD patients) and subpleural consolidation with patchy ground glass infiltrates is seen in organizing pneumonia (OP) (one-tenth of RA-ILD patients) [69]. In contrast to NSIP, UIP is a pattern most often found in men and smokers [70]. UIP demonstrates the same histological subtype as the most common, progressive fibrosing lung disease, idiopathic pulmonary fibrosis (IPF), and although survival is improved compared with IPF, survival is poor compared with NSIP/OP, with 5-year survival estimated to be <50% [71].

Although one small study found no association with anti-CCP positivity [72], we demonstrated a strong association with anti-CCP positivity in 94% and RF in 89% of patients with RA-ILD [73]. This is once again significantly higher than in patients with RA alone and is in agreement with findings in a large cohort of Greek RA-ILD patients [74]. Furthermore, Harlow et al. [75] identified autoantibodies specific to RA-ILD that react to citrullinated heat shock proteins (Hsp90/Hsp90). The role of these autoantibodies in the pathogenesis of RA lung disease warrants further investigation, particularly given that raised levels of Hsp90 (not known if citrullinated) have been reported in patients chronically infected with Pseudomonas aeruginosa, a common pathogen isolated from the sputum of bronchiectasis patients [76].

Smoking is associated with the physiological abnormalities consistent with RA-ILD [73, 77]. Genetic features in RA-ILD are associated with HLA-DRB1*1501/*1502 [64] but not with HLA-DRB1 SE alleles; indeed, a negative association with HLA-DRB1 SE [78] was found in one Japanese cohort. There is evidence suggesting an association between anti-CCP positivity, RF positivity and RA-ILD. But genetic and environmental associations appear to be different from those seen in bronchiectasis and the mechanism driving RA autoantibody production is unknown.

Obstructive disease of smaller airways
Mori et al. [59] reported the prevalence of obstructive dysfunction in small airways in RA patients. Small airway obstruction in patients with RA estimated on the basis of decreases in forced expiratory flow of 25–75% of vital capacity (FEF25–75) varies among studies at 8–65%. Mori et al. [59] undertook pulmonary function testing and HRCT on 189 consecutive RA patients. HRCT revealed 8% of patients with a small airway obstruction pattern. This finding was not explained by smoking, as only 13% of these patients had ever smoked. Their definition of obstructive dysfunction of small airways was an FEF25–75 value >1.96 residual S.D. below predicted values and was observed in all those RA patients with HRCT, providing evidence of a small airway obstruction pattern on HRCT. In those with no abnormal HRCT patterns in terms of classical bronchiolitis or interstitial pneumonia, parenchymal micronodules were observed in 16% of RA patients, bronchial wall thickening in 8% and bronchial dilatation in 30%. A small airway obstruction pattern was observed in one-third of these RA patients. These subtle HRCT findings are important, as a Swedish study of 105 RA patients with very early untreated RA disease demonstrated that the majority of the ACPA-positive RA patients (63%) compared with ACPA-negative RA patients (37%) had similar HRCT lung abnormalities. These differences were independent of the smoking status of the RA patients. Additionally, on biopsy there was increased lung tissue citrullination in ACPA-positive vs ACPA-negative RA patients. Importantly, ACPA levels were higher in BAL than in sera of ACPA-positive patients, suggesting lung ACPA production in these patients [55].

Are RA autoantibodies associated with lung pathology a sign of problems to come?
The associations between RA-related lung diseases and RA autoantibody positivity are unlikely to be coincidental and cannot be explained by smoking alone. Several studies have found increased levels of RA autoantibodies in patients without inflammatory arthritis but with lung diseases recognized to be associated with RA. Notably, increased RA autoantibody positivity is present in patients with ILD [79]. A further study identified similar findings in chronic obstructive pulmonary disease (COPD). Although COPD is not a primary RA-related lung disease, its prevalence does increase in the RA population [80] and ACPA positivity in patients with COPD has shown that heavy smokers with COPD are more prone to ACPA production compared with heavy smokers without COPD and a non-smokers control group [9]. This increase was confirmed by another study that demonstrated a 5.2% prevalence of anti-CCP in patients with COPD [81]. Genetic associations have not been reported specifically for RA patients with COPD.

Supporting the possibility that lung pathology drives the production of RA autoantibodies, HRCT studies have demonstrated increased prevalence of subclinical pulmonary abnormalities in newly diagnosed RA patients [82]. Furthermore, HRCT airway abnormalities in RA autoantibody-positive subjects without inflammatory arthritis have been shown to be similar to patients with early RA and significantly higher than RA autoantibody-negative controls [82].
Hypotheses for the potential role of lung pathology in the development of RA

The respiratory tract interfaces with the external environment and is bombarded constantly by microbes and allergens [83]. A robust immune defence is essential (Table 1), yet suppression of responses is required for low-level bacterial exposure that may be normal or can be cleared through innate immunity, e.g. mucociliary escalator. A breakdown in immune tolerance following external environmental stimuli such as smoking or infection could potentially trigger autoimmunity and consequent development of RA.

Cigarette smoke and iBALT

The study of cigarette smoke and genetic susceptibility has led to one of the most researched and best recognized conceptual hypotheses for the pathogenesis of ACPA-positive RA:

(i) Environmental triggers at sites distant from the joints, e.g. smoking or infection, results in increased citrullination of peptides.

(ii) Susceptible individuals include carriers of HLA-DRB1 SE±PTPN22 R620W.

(iii) Resultant B cell activation in susceptible individuals leads to ACPA production. ACPA response evolves over time with an increasing number of ACPA specificities (e.g. anti-CEP1) and class switching to IgM ACPA isotypes.

(iv) A second hit in the synovium (trauma/local inflammation) results in citrullination of synovial peptides with or without synergistic interaction with RF, resulting in the development of chronic autoimmune-driven inflammation presenting clinically as ACPA-positive RA.

(v) There is evidence suggesting that iBALT has a key role in pulmonary adaptive immunity. iBALT has been shown to express endothelial and lymphocyte adhesion molecules with the potential to recruit memory and effector lymphocytes [84]. Moreover, plasma cells in iBALT are known to be capable of generating RF and ACPA [56]. Increased prevalence of iBALT occurs in the lungs of smokers [85] and in rheumatoid-associated pulmonary disease, particularly RA-ILD [56]. Indeed, it is possible that ACPA production thorough B cell activation may be a consequence of adaptive immunity generated by iBALT.

Infections

Recurrent infection is a clinical feature of COPD and bronchiectasis. Bronchiectasis patients have a higher risk of ACPA-positive RA despite a high prevalence of never smokers. There are numerous mechanisms by which pulmonary infections may be influential in the pathogenesis of ACPA-positive RA.

Microbial molecular mimicry

P. gingivalis is an extensively studied bacterium in the pathogenesis of RA. Indeed, ACPAs to CEP-1 have been shown to react with citrullinated P. gingivalis enolase [50], suggesting a role for microbial mimicry. P. gingivalis is typically isolated from the oral mucosa and is not a recognized pulmonary pathogen. Streptococcus pneumoniae and Streptococcus pyogenes and Streptococcus pneumoniae are both pulmonary pathogens that, like P. gingivalis, express α-enolase [86, 87]. Immunity to and citrullination of this bacterial α-enolase could, by molecular mimicry, lead to the development of ACPA.

Neurone-specific enolase

Associations with ACPA-positive RA have been found predominantly for antibodies to α-enolase. Enolase exists in three recognized isoforms: α-enolase is found in most human tissues, whereas β-enolase is predominantly found in muscle tissues and γ-enolase or neurone-specific enolase is found in neurone and neuroendocrine tissues [88]. Pulmonary neuroendocrine cells are thought to play a role in airway response to hypoxia [89] and in the regulation of epithelial cell growth and regeneration [90]. Pulmonary neuroendocrine tissue is hyperplastic in certain conditions, and during infections pulmonary neuroendocrine tissue proliferates, especially in ILD [91], bronchiectasis [92] and smoking/COPD [93]. Neurone-specific enolase is expressed by pulmonary neuroendocrine cells and has an 88% similarity to α-enolase. It is therefore possible that in the pathogenesis of RA, ACPAs develop initially to citrullinated neurone-specific enolase on pulmonary neuroendocrine tissue.

Neutrophil extracellular traps

The release of neutrophil extracellular traps (NETs) via a novel form of cell death (NETosis) occurs in neutrophil responses to bacteria. Increased NETosis was recently observed in the blood circulation and synovial fluid of RA patients compared with healthy controls [94]. NETosis levels were found to correlate with ACPAs and with systemic inflammatory markers [94]. ACPAs and/or RF were shown to enhance NETosis and alter the distinct content of citrullinated peptides expressed by NETs [94]. Furthermore, IL-17 and TNF-α were shown to induce NETosis and NETs were shown to augment synovial inflammatory responses in fibroblasts through induction of IL-6, IL-8, chemokines and adhesion molecules [94]. These observations suggest increased NETosis may have a key role in the breakdown of immune tolerance to citrullinated peptides and in the pathogenesis of RA. It is possible that increased NETosis may be one of the mechanisms by which bronchiectasis patients are at particularly increased risk of developing ACPA-positive RA.

Conclusions

We have detailed the evidence and potential hypotheses (Fig. 3) by which autoimmunity to citrullinated peptides...
and the development of ACPA-positive RA may be initiated within the respiratory system.

However, RA is a complex inflammatory disease, and intricate molecular mechanisms with multiple environmental and genetic risk factors contribute to the clinical spectrum of RA. Although focusing on ACPA-positive RA has facilitated greater understanding of the pathogenesis, including recognized genetic and environmental risk factors and a key role of adaptive immunity. Central questions defining the research agenda include, at an immunological level, how do antigen-presenting cells, T cells and B cells interact to promote immune responses to citrullinated antigens? How does the immune response to citrullinated antigens evolve over time to result in the clinical presentation of RA and can this be targeted with therapeutic interventions to halt progression? Is the lung a site for the development of such immune responses?

Prospective studies including genetic and environmental associations, tissue studies at the immunohistochemical level, better understanding of the lung microbiome in health and disease and the identification of novel autoantigens are critical for our future understanding of ACPA-positive RA pathogenesis. The lung is an immunologically active organ and should be a focus for further research as a potential primary initiating site for ACPA-positive RA.

Rheumatology key messages

- Autoimmunity to citrullinated peptides plays a key role in the development of ACPA-positive RA.
- High levels of RA autoantibody positivity are recognized in RA-related lung diseases.
- The lung should be a focus for further research as a potential primary initiating site for ACPA-positive RA.

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