Review

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The lung may play a role in the pathogenesis of rheumatoid arthritis

Multiple studies have identified strong associations between the lung and rheumatoid arthritis (RA). Such studies identify a high prevalence of lung disease, both airways and parenchymal disease, in subjects with clinically classifiable RA. It has been suggested that lung disease in RA results from targeting of the lung from circulating autoimmunity or other factors such as medications. However, findings that lung disease, specifically inflammatory airways disease, and lung generation of autoimmunity can be present before the onset of joint symptoms suggest that immune reactions in the lung may be involved in the initial development of RA-related autoimmunity. Herein we review these issues in detail, as well as outline a potential research agenda to understand the natural history of lung involvement in RA and its relation to the overall pathogenesis of RA.

Keywords: lung disease • pathogenesis • preclinical autoimmunity • rheumatoid arthritis

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Expert commentary

The primary clinical manifestation of rheumatoid arthritis (RA) is joint disease, although RA-related injuries to other tissues, including the lung, have long been recognized in RA. This extra-articular tissue involvement has typically been thought of as being a target of RA-related autoimmunity, or an effect of medications or secondary infections. However, emerging data suggest that the lung may play a role in the initiation of RA, and not just be a target of autoimmunity or other processes, with this initiation perhaps due to gene and environmental interactions at the mucosal surface of the lung. In particular, the identification of a 'preclinical' period of RA development during which there are elevations of circulating RA-related autoantibodies in absence of joint disease, as well as unique studies of human cohorts who are at risk for future RA, have supported the concept that RA may be initiated outside of the joints and in the lung.

However, given that there are multiple anatomic compartments of the lung that have differing immunologic and physiologic properties, and there are multiple manifestations of RA-related lung disease, it still may be that some aspects of lung disease in RA are a result of secondary immune-related injury from systemic autoimmunity. For example, the airways may be a site of initiation of autoimmunity in RA, while the parenchyma may be a target of circulating autoimmunity that leads to nodules or interstitial lung disease (ILD).

As the field moves forward towards a greater understanding of the role of the lung in RA, it will likely require new approaches to investigate RA-related autoimmunity. These approaches may include novel methods to assess generation of autoimmunity at specific anatomic sites, including cellular and humoral responses, and systemic and local inflammation. These methods will also likely need to include ways to safely obtain informative mucosal-based biospecimens. Also needed are new tools to investigate potential triggers for disease including advanced genetic and microbial analyses. Perhaps most importantly, novel cohorts are

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needed that can be used to evaluate the natural history and pathogenesis of RA, and offer new perspectives on how RA develops including a focus on the lung. Importantly, advances in understanding this area may lead to improved means of treating lung disease in patients with RA, as well as ultimately develop preventive strategies for RA that may target the lung.

Overview of RA

RA is a systemic inflammatory autoimmune disease that primarily affects the joints. It affects approximately 0.5–1% of the population, and predominately affects women over men, in a ratio of approximately 3:1 [1]. The specific etiology of RA is not known, however there are multiple genes associated with increased risk for disease, with MHC alleles containing protein sequences termed the 'shared epitope' having the strongest association with disease [2]. In addition, multiple environmental factors have been associated with RA, including dietary and nutritional factors, and occupational exposures (including exposure to dust), with the strongest environmental factor being exposure to tobacco smoke, which has been estimated to explain 20–30% of RA risk [2,3].

A clinical diagnosis of RA relies on the presence of persistent inflammatory arthritis that can be classified as RA when that inflammatory arthritis meets certain criteria as defined in the 1987 American College of Rheumatology (ACR) Revised Criteria [4], or the 2010 ACR/European League Against Rheumatism Classification Criteria [5]. Of note, inflammatory arthritis meeting these classification criteria will be termed 'classifiable' RA herein. Furthermore, RA is characterized in the majority of cases by elevations of RA-related autoantibodies (see Table 1), including rheumatoid factor (RF) or antibodies to citrullinated protein antigens (ACPAs), although 20–30% of patients may be negative for these autoantibodies and be designated 'seronegative' RA [6,7].

While RA is diagnosed when a patient presents with the signs and symptoms of inflammatory arthritis, it is now well established that elevations of autoantibodies and inflammatory markers can be present on average 3–5 years (and as long as 15+ years) prior the onset of joint disease [6–7,14–17]. This period of autoimmunity and inflammation in absence of joint disease is currently termed the 'preclinical' period of RA, and a growing understanding of this phase of RA development has revolutionized the understanding of the pathogenesis of RA. Importantly, because circulating autoimmunity is present in many cases in absence of physical examination [18], imaging [19] or synovial biopsy [20] evidence of joint inflammation during the preclinical period of RA development, it strongly suggests that RA is initiated at an anatomic site outside of the joints.

Lung disease is highly prevalent in clinically classifiable RA

While the joints are the predominant organs targeted by immune and inflammatory responses in RA, other tissues can be affected as well. This review will focus on pulmonary involvement in RA, but extra-articular involvement in RA can additionally include cutaneous, ocular, cardiac, neurologic and hematologic manifestations (reviewed in [21,22]).

In order to understand the types of lung disease that can be present in RA, differences should be considered between anatomic compartments of the lung including the airways, parenchyma, pulmonary vasculature and the pleura [23]. The airways of the lung are defined as the passageways through which air moves in the lung and include the bronchi, bronchioles and terminal bronchioles [24]. The lung parenchyma is defined as the functional tissue involved in gas transfer that includes the alveoli, and the interstitium between the alveoli and lung capillaries. The lung vasculature includes the pulmonary arteries and veins that carry blood for gas exchanges as well as the smaller bronchial arteries and capillaries that nourish the airways, lung tissue and alveoli. Because the pulmonary vasculature is a key component of gas exchange, it is sometimes considered to be a part of the lung parenchyma. However, clinical disease affecting the pulmonary vasculature is distinct from ILD; therefore, herein, pulmonary vascular involvement will be considered separate from parenchymal lung involvement. Finally, the lung pleura is the thin membranous lining surrounding the lung.

Each of these regions of the lung have unique anatomic and functional abnormalities as well as clinical characteristics associated with RA that are summarized in Table 2. For example, airways disease pathologically involves inflammation of the small or large airways (including rarely the cricoarytenoid joints in RA [25]), and on high-resolution computed tomography (HRCT) can include bronchial wall thickening, bronchiectasis, centrilobular opacities consistent with bronchiolitis or mosaic attenuation on expiration consistent with abnormal air trapping. In airways disease, physiologic testing of the lungs through modalities such as pulmonary function testing (PFT) can demonstrate obstructive pulmonary physiology, manifested by reductions in forced expiratory airflows [24]. The exact prevalence of airways disease in RA varies with these diagnostic modalities. With PFT, the prevalence of airways disease in classifiable RA ranges from 8 to 36% [8,26–30]. However, with more sensitive tests such as HRCT, the majority of studies suggest approximately 60–80% of

Table 1. Autoantibodies identified in rheumatoid arthritis and associations with the lung disease in rheumatoid arthritis.

Autoantibody	Association with lung disease in RA	Ref.
RF	Presence of RF may be associated with increased risk for parenchymal lung disease in RA	[8]
ACPAs	Higher ACPA levels and increased number of high level ACPAs are associated with more severe parenchymal lung disease in RA	[9]
Antibodies to citrullinated HSP90	Anti-HSP90 antibodies are highly specific for parenchymal lung disease in patients with RA	[10]
Antibodies to MCV	In subjects with RA, anti-MCV IgA positivity was higher in current and former smokers compared with never smokers	[11]
Antibodies to CarPs and PAD4	Anti-CarP and Anti-PAD4 antibodies are highly specific for RA and associated with more severe joint disease. However, there are no currently published studies evaluating their association with lung disease in RA	[12,13]

ACPAs: Antibodies to citrullinated protein antigens; CarP: Carbamylated protein; MCV: Mutated citrullinated vimentin; RA: Rheumatoid arthritis; RF: Rheumatoid factor.

RA subjects have clinical or subclinical airways disease. Parenchymal disease can be manifested on imaging studies (e.g., HRCT) by nodules, alveolar infiltrates that appear as ground glass opacities, or fibrotic disease that includes reticulation and/or honeycomb change, and identified on PFT through restrictive physiology and decreased diffusion capacity [24]. Depending on the subject demographics and ascertainment method, the prevalence of parenchymal lung disease in subjects with RA is reported as low as 7% up to as high as 79% [8,27–29]. Pleural disease can include mild inflammation identified by pleural thickening on imaging, or severe inflammation with pleural effusions that can readily be identified on physical examination or imaging. Pleural disease can be identified on imaging in up to 50% of patients with classifiable RA [28].

In aggregate, these studies consistently demonstrate a high prevalence of some form of lung disease in subjects with classifiable RA [8,26–32]; however, there is significant variability in the prevalence rates reported. This variability is likely due to a number of factors: heterogeneity of patients studied (e.g., varying RA disease duration, disease severity, autoantibody positivity or risk factor exposures such as medications or smoking); heterogeneity of each type of lung disease; differences in subject recruitment (e.g., population-based studies vs subjects undergoing lung imaging for clinically significant symptoms); and different diagnostic modalities for assessment of lung disease (e.g., chest radiograph vs PFT vs HRCT). For example, multiple studies demonstrate HRCT is more sensitive than PFTs in the diagnosis of airways and parenchymal lung disease including studies of subjects with severe asthma as well as RA-associated ILD [30,33–34]. Further adding to this variability is the finding that the majority of lung disease in patients with RA is asymptomatic and considered 'subclinical' lung disease [28,32]. Specifically, in a recent study by Chen and colleagues in which HRCT was utilized, 61% of subjects with classifiable RA (mean disease duration over 4 years) had evidence of lung disease, and 90% of those with lung disease were asymptomatic [32].

In addition, the timing of assessment of lung disease in RA seems to impact the frequency and in particular the types of lung disease that are seen. Several studies demonstrate a higher prevalence of airways disease in early classifiable RA [29,31], as well as preclinical RA discussed in detail below [26,35]. These studies include work by Wilsher and colleagues who identified that airways disease on HRCT was more prevalent than parenchymal lung disease in subjects with early classifiable RA (82% with airways disease vs 23% with parenchymal disease) [29]. Also, in a study by Metafratzi and colleagues that utilized HRCT in subjects with early classifiable RA (disease duration less than 1 year), there was a higher prevalence of airways disease (69% with air trapping and 58% with bronchiectasis) compared with parenchymal lung disease (35% with ground glass opacities) in the early stages of RA [31]. However, predominantly airways disease in early RA has not been found by all investigators; in particular, Reynisdottir and colleagues evaluated disease-modifying antirheumatic drug (DMARD)-naive subjects with early classifiable RA (joint symptom duration less than 16 months, median 6 months) utilizing HRCT and found a higher prevalence of parenchymal lung disease (54% with evidence of parenchymal disease compared with 42% with evidence of airways disease) [27]. In this study, the higher prevalence of parenchymal disease may be related to different classification of type of lung disease in these patients or other factors related to the types of subjects studied. Indeed, early RA subjects without a

Table 2. Types of lung disease in rheumatoid arthritis.

	Airways	Parenchymal	Pleural	Vascular
Radiographic findings	Bronchial wall thickening Bronchiectasis Centrilobular opacities Air trapping	Nodules Ground glass opacities Reticulation Honeycombing	Pleural thickening Pleural effusion	Mosiac attenuation Pulmonary artery enlargement
PFT findings	Obstruction Air trapping/elevated residual volume	Restriction Decreased diffusion capacity	Reduced lung volumes	Decreased diffusion capacity
Associated clinical disease	Emphysema Bronchitis Asthma	Interstitial lung disease	Pleuritis Pleural effusion	Pulmonary artery hypertension

PFT: Pulmonary function testing.

reported history of smoking had a higher prevalence of airways disease compared with parenchymal disease (75 vs 32%, respectively).

Differing risk factors & roles of airways & parenchymal lung disease in RA

There are few data available regarding risk factors for specific forms of lung disease, although risk factors for symptomatic lung disease in RA in general include longer duration of articular disease, tobacco smoke exposure, male sex, exposure to certain medications (e.g., methotrexate), the presence of RF and, in some studies, the presence of ACPAs and high levels of autoantibodies (although the two latter associations are variable across studies) [36]. In addition, Gochuico and colleagues have found that cigarette smoking and methotrexate were risk factors for progression of lung disease in RA patients with initially minimally symptomatic ILD at baseline [37].

However, some data are available that support differing risk factors for differing types of lung disease. Specifically, in the study mentioned above by Wilsher and colleagues [29], serum ACPA positivity was associated with presence of airways disease, whereas serum RF positivity was associated with parenchymal disease. Furthermore, Mori and colleagues studied 356 Japanese patients with RA and found similar results; specifically, high levels of RF were associated with parenchymal disease/ILD, while high levels of ACPAs were associated with airways disease [8]. In addition, they found that the HLA DRB1*1502 was associated with parenchymal disease/ILD, but nearly protective for airways disease.

Although other studies have found serum ACPAs to be associated with parenchymal lung disease in early RA [27], both the Wilsher and Mori studies suggest the potential that different forms of lung disease in RA may be associated with different mechanisms of pathogenesis. While speculative, this leads to the concept that certain autoantibodies (e.g., RF or ACPA) may be associated with different patterns of lung injury, or even that different autoantibodies may be generated in different areas of the lung. It is reasonable that there may be differing risk factors for airways versus parenchymal disease in RA because the mucosal surface of the airways directly interfaces the immune system and inhaled environmental factors, while, as discussed below, the parenchyma of the lung may be more exposed to circulating factors [38]. In particular, airways disease in early RA could represent gene and environmental interactions that lead to localized airways inflammation, or as discussed below perhaps even a site of initial immune dysregulation in RA. While parenchymal lung disease in RA may also arise from inhaled factors if they are selectively retained in the alveoli such as in occupational lung disease [39], it may be more likely that parenchymal disease in RA develops from other biologic mechanisms, especially since the lung parenchyma is exposed to the systemic circulation. Such mechanisms may include RA-related immune complexes that become trapped when circulating through the lung, RA-related autoantibodies generated elsewhere that may be cross-reactive with proteins in the lung or nonautoimmune inflammatory reactions to inhaled factors that result in parenchymal disease. Of note, these mechanisms may not be mutually exclusive within an individual, and they may also develop at different time points in the natural history of RA. It is also possible that inflammation initiated at one site of the lung can 'spread' to other sites. For example, there is an emerging hypothesis in idiopathic pulmonary fibrosis that the site of initial pathology is the airways, with parenchymal inflammation developing as a result of spread of inflammation from the airways over time [40,41]; similar processes may occur in RA.

Therefore, in order to determine how each type of lung disease is involved in the overall pathogenesis of

RA, natural history studies of RA-related lung disease are needed that include prospective studies of subjects that progress from preclinical to late stages of disease. Such natural history studies can advance our understanding of how airways disease and parenchymal disease are related in RA and in particular address the question of whether RA-related lung disease may start in the airways and move elsewhere, and risk factors that may be more associated with specific forms of lung disease.

Additionally, natural history studies of lung disease could facilitate biomarker discovery in RA-related lung disease, and help identify at what stage of lung disease certain biomarkers are most relevant. If certain biomarkers are found to be associated with certain types of lung disease, this finding may yield insights into the pathogenesis of disease at each site within the lung. Of note, many of the studies evaluating the biologic relevance and predictive nature of serum biomarkers for RA-related lung disease have focused on biomarkers that have been previously identified in RA-related joint disease [9,26,42]. For example, Giles and colleagues found that higher serum ACPA levels and greater number of high level ACPAs were associated with more severe parenchymal lung disease in subjects with classifiable RA [9], but the ACPA array utilized in this study was based on citrullinated proteins/peptides specific for RA-related joint disease [43]. While these ACPAs may also be important in lung disease, novel lung-disease specific biomarkers may provide improved insight into the biologic mechanisms of lung disease development and progression in RA. Gochuico and colleagues found that higher levels of IFN-γ and TGF-β were present in bronchoalveolar lavage (BAL) fluid from subjects who had progressive RA-ILD compared with those with stable disease [37]. Additionally, Harlow and colleagues have identified a specific ACPA to citrullinated HSP90 that was highly specific (>95%) although minimally sensitive (20–30%) for RA-associated ILD[10]. Additional research in this area is therefore needed to identify biomarkers that are able to predict progression of RA-related lung disease, and help us to understand mechanisms of disease development.

Lung disease, & in particular airways disease, may occur prior to the onset of articular RA

Importantly, while lung disease in RA is usually identified after the onset of joint disease, there are data supporting that lung disease may precede the onset of clinically apparent joint disease. In particular, a 2009 study by Gizinski and colleagues described four patients with ILD who had serum RF and ACPA positivity in the absence of arthritis, with one of these subjects later developing classifiable articular RA [44]. Furthermore, a 2012 study by Demoruelle and colleagues found a significantly higher prevalence of airways disease present on HRCT imaging in arthritis-free subjects with serum RA-related autoantibodies compared with autoantibody negative controls (76 vs 33%, respectively), with two of these subjects with airways disease and autoantibody positivity developing classifiable RA within 2 years of their lung evaluations [26]. Of note, this association was independent of smoking, and all subjects were without inflammatory arthritis based on detailed clinical joint examination, including a subset who also underwent joint MRI that demonstrated absence of synovitis.

Another recent study by Fischer and colleagues evaluated the prevalence of different types of lung disease in arthritis-free subjects with serum ACPA positivity and symptomatic lung disease [35]. In this study, HRCT imaging identified that the most common finding associated with ACPA positivity in absence of synovitis was airways disease, identified in 81% of subjects compared with parenchymal lung disease identified in 41%. A subset of these ACPA positive arthritis-free subjects underwent clinically indicated bronchial biopsy, and of those subjects, 90% had histopathologic evidence of airways inflammation with lymphoplasmacytic and chronic inflammation. Importantly, three of these subjects with airways disease and autoantibody positivity developed classifiable RA within 3 years of follow-up. Although generalizability is limited because these subjects had symptomatic lung disease, this study confirms that the high prevalence of airways abnormalities seen on imaging in the preclinical period of RA represent pathologic airways inflammation.

Taken together, these studies demonstrate that lung disease, and in particular, inflammatory airways disease is associated with serum RA-related autoantibodies in absence of joint inflammation. Of particular interest, these studies report several subjects that developed synovitis clinically classifiable as RA several months after lung imaging. In these subjects, inflammatory airways disease clearly preceded the onset of joint inflammation strongly suggesting the lung is either a site of initial inflammation and autoimmunity or a very early target of inflammation and autoimmunity during the preclinical period.

RA may originate at the airways mucosa

As discussed above, the preclinical period of RA development during which there are elevations of disease-related autoantibodies in absence of joint inflammation suggests that RA may be initiated outside of the joints. The exact anatomic site where RA originates is

currently unknown; however, several lines of evidence suggest that RA may originate at a mucosal surface [7,11,45–48]. At mucosal surfaces, exposure to environmental factors such as bacteria can induce local as well as systemic IgA responses [49,50], and such a naturally occurring biologic process lends opportunity for a potential dysregulated immune response that could result in the development of autoimmunity in susceptible individuals. Although there are multiple mucosal surfaces that are potential sites for development of RA (e.g., gingival, gastrointestinal, genitourinary), the data mentioned above that identify a high prevalence of airways disease during the preclinical period and lung disease preceding joint disease in some subjects that develop classifiable RA suggest that the lung, specifically the airways mucosa, may be an important and prevalent site where autoimmunity in RA originates [26,35,44].

Although it is possible that the associations of airways inflammation and systemic RA-related autoantibodies are related to the lung being targeted by autoimmunity generated elsewhere, there is also compelling data that RA-related autoimmunity is actually generated within the lung. In particular, several studies of subjects with classifiable RA have identified local RA-related autoantibodies present in the lung [27,51–53]. Specifically, a study by Rangel-Moreno and colleagues found elevated ACPAs in BAL fluid of subjects with long-standing seropositive RA and RA-related ILD [51], and a more recent study by Reynisdottir and colleagues identified elevated IgA- and IgG-ACPAs in BAL fluid from subjects with early classifiable RA, the majority of whom had underlying lung disease on HRCT [27]. In addition, a study by Schiotz and colleagues used sputum studies to demonstrate the generation of RF and antinuclear antibodies in the lungs of patients with cystic fibrosis [54], further supporting that the lung can be a site of generation of autoimmunity.

Furthermore, if the lung is a site of initiation of RA, local lung generation of RA-related autoimmunity should be present during the preclinical period. This hypothesis was tested in a recent study by Willis, Demoruelle and colleagues that utilized induced sputum samples to evaluate local generation of RA-related autoantibodies in the lung fluid of subjects with classifiable RA as well as subjects at risk for future RA [53]. In this study, subjects with early classifiable RA (disease duration less than 12 months) had significantly higher levels of sputum ACPAs (as measured by anticyclic citrullinated peptides) and RF isotypes (IgA, IgM and IgG) compared with healthy control subjects suggesting these sputum RA-related autoantibodies are specific for classifiable RA. In addition, 65% of subjects in the preclinical period of RA (i.e., serum RA-related autoantibody positivity in absence of clinical synovitis) had at least one RA-related autoantibody present in the sputum, and in arthritis-free seronegative subjects at elevated risk for future RA based on family history of RA, nine of 23 (39%) had at least one RA-related autoantibody present in their sputum in absence of serum autoantibody positivity. This study also determined the ratio of autoantibody level to total Ig level in sputum and serum, and with this method, they found that in arthritis-free subjects with sputum autoantibody positivity, the autoantibody:Ig level was higher in sputum compared with serum suggesting that in this subset of subjects at risk for RA, these RA-related autoantibodies appear to be generated in the lung.

Additional factors suggesting RA-related autoantibodies may be generated in the lung

Exposure to tobacco smoke has long been associated with increased risk for development of clinically classifiable RA as well as RA-associated lung disease [3,36,55]. In addition, recent studies of subjects with smoking associated chronic airways disease, specifically chronic obstructive lung disease (COPD), without joint inflammation have identified an increased prevalence of serum ACPA positivity further supporting that the lung may be a site of generation of RA-related autoimmunity [55,56]. Specifically, Ruiz-Esquide and colleagues evaluated subjects without RA who had a heavy smoking history and COPD, and in these subjects, they found a higher prevalence of serum ACPA positivity (4–7%) compared with controls without a history of smoking (2%) [56].

Mechanisms that may be involved in the generation of RA-related autoimmunity in the lung

The above findings of lung disease, and in particular airways disease prior to the onset of joint inflammation in RA, and the generation of RA-related autoantibodies in the lung, strongly suggest that the lung is a site of initiation of RA-related autoimmunity. However, if this is the case, what is driving the generation of that autoimmunity?

Tobacco smoke exposure

As discussed above, tobacco smoking is associated with risk of RA, but the exact mechanisms by which this occurs are unknown. A 2006 study by Klareskog and colleagues found a strong association between current smoking and the presence of citrullinated peptides in the lung in subjects without RA [3]. In 2008,

Makrygiannakis and colleagues found a similar association of increased presence of citrullinated peptides in the BAL fluid of smoking subjects without RA [57]. In addition, this study found that the enzymes responsible for citrullination of peptides in humans, PAD-2 and PAD-4, were highly expressed in BAL fluid and lung tissue of smokers, although only PAD2 expression was higher in smokers than nonsmokers. These studies suggest smoking could induce local lung generation of citrullinated peptides, and this may be involved in the initiation of RA in the lung given that citrullinated peptides are the autoantigen target of ACPAs. In addition, multiple studies linking the presence of RF with cigarette smoking and lung disease, even in absence of RA, suggest that tobacco smoke may also affect generation of this autoantibody system [58,59].

However, these studies demonstrating elevated citrullinated proteins in the lung did not evaluate whether the presence of smoking-associated citrullinated peptides were associated with local or systemic RA-related autoimmunity. Since it is well known that any inflammation can result in the generation of citrullinated peptides [57,60], it may be that the development of citrullinated peptides alone is not enough to trigger an autoimmune response. Such a possibility was proposed in a study by Baka and colleagues who identified lung tissue elevations of citrullinated peptides and PAD-4 in lung tissue from subjects with lung cancer, although few had serum ACPA elevations [61]. These data suggest other factors may be necessary to generate an autoimmune response to citrullinated peptides present in the lung. Since there is a known association between certain HLA alleles, serum ACPAs and smoking in RA, it may be that an important factor in generation of autoimmunity to citrullinated proteins in the lung is an appropriate genetic background [3,62–63]; however, as many subjects with ACPAs do not have known risk alleles for RA [64], the presence of additional inflammatory factors may also be necessary to generate autoimmunity to citrullinated proteins. Further studies are needed to understand these additional factors that may include genetic factors or cumulative environmental factors.

Another potential interesting mechanism for the role of smoking and autoantibody generation in RA was recently proposed by Newkirk and colleagues [55]. In this study, they evaluated serum anti-HSP-70 autoantibodies, and they found IgM-HSP-70 autoantibodies were associated with presence of chronic lung disease independent of smoking. However, they found that class switching to IgG-HSP-70 autoantibodies was associated with chronic cigarette smoke exposure. Furthermore, in a murine model, IgG-HSP-70 autoantibodies induced RF-expressing B cells to generate increased RF-IgM. Based on these data the authors concluded that smoking may be necessary for class switching of anti-HSP-70 autoantibodies to an isotype that can in turn drive the production of the RA-related autoantibody RF. It was also of interest in this study that the length of time exposed to cigarette smoke was important as only chronic and not acute smoking exposure was associated with serum RF positivity in mouse models. This is supported by additional data demonstrating only smoking greater than 10 pack-years was associated with increased serum ACPA positivity [65].

Microorganisms

While smoking is a strong environmental risk factor for RA, with some estimates that it explains up to 35% of risk for seropositive RA [63], not all subjects that develop RA have a history of smoking, implying that other environmental factors may contribute to lung inflammation and autoimmunity in RA. Emerging data suggest RA may be triggered by microorganisms and in particular certain species of bacteria [66–69]. These data include recent studies that have identified associations between RA and *Prevotella* species and *Porphyromonas gingivalis* [70–72]. Importantly, recent culture-independent methodologies have led to comprehensive detection of commensal bacteria identified by their DNA sequences, even in absence of overt pathologic infection [73–76]. These advances have led to identification of specific bacterial communities in the lung [77,78]. Of note, bacteria-specific mechanisms are known to influence the development of innate and adaptive immunity at mucosal surfaces [79,80], and also likely play a role in development of autoimmunity through mechanisms such as molecular mimicry, bacteria-induced autoantigen generation and immune regulatory effects [81–85].

Of interest regarding potential mechanisms for RA initiation in the lung, bacteria associated with RA such as *Prevotella* and *Porphyromonas gingivalis* have been identified in the lung along with other bacteria associated with lung inflammation [77,78]. *Porphyromonas gingivalis* is found to contain a bacterial PAD that citrullinates the human proteins fibrinogen and α-enolase [70,86–87]. Antibodies to citrullinated fibrinogen and α-enolase are identified in the preclinical period of RA [17], suggesting a potential mechanism by which a certain bacteria may lead to RA-related autoimmunity through specific metabolomic effects (e.g., protein citrullination). Because of the close proximity of the lung to the oral cavity, it may be that organisms predominately located in the oral cavity, such as *Porphyromonas gingivalis*, move to the lung and cause pathology in the airways.

While identifying a single risk factor (e.g., tobacco smoke or a specific organism) for RA that may impact the mucosa is an attractive concept, it should be noted that it may be multiple environmental factors that contribute to development of RA. For example, cigarette smoking may change the lung microbiome resulting in a specific bacterial community that can then trigger autoimmunity, generalized inflammation resulting from smoking or bacteria could result in an inflammatory milieu in the lung making individuals more susceptible to develop RA, or smoking and specific lung microbiota could act simultaneously to cross a threshold that triggers autoimmunity in RA. Certainly, further studies are needed that include investigations to understand these complex relationships and identify whether specific microbiota in the lung are directly associated with generation of RA-related autoantibodies. In addition, the influence of microbiota at other mucosal sites must be taken in to account as it relates to the pathogenesis of RA.

Host mechanisms for developing autoimmunity in the lung: inducible bronchus associated lymphoid tissue

As discussed, inhaled environmental factors such as smoking and/or bacteria may be involved in mechanisms of generation of autoimmunity in RA. Yet, it is unknown how these factors may directly interact with the immune system at the cellular level to generate RA-related autoimmunity in the lung. However, inducible bronchus associated lymphoid tissue (iBALT) is one possible mechanism based on its immunologic features and associations with RA.

iBALT is an ectopic lymphoid tissue that contains follicular aggregates of T and B cells, and antigen-presenting follicular dendritic cells [38,88]. It represents a lung-specific immune response that can generate local antibodies in the lung. In addition, BALT is not preprogrammed and therefore not present in healthy human lung tissue. However, it can be induced locally in direct response to infection, inhaled antigens or inflammation at the airways mucosa [89]. Of interest in RA, Rangel-Moreno and colleagues demonstrated that in lung tissue from subjects with chronic RA-related lung disease, iBALT was present in increased prevalence, size and was more organized than the lymphoid follicles present in subjects with other forms of chronic lung disease [51]. In addition, these areas of iBALT in subjects with RA included plasma cells generating RA-related autoantibodies that were associated with elevations of ACPAs in the lung fluid. Thus, iBALT may represent a mechanism by which RA-related autoimmunity is generated locally in the lung. Similar areas of mucosal associated lymphoid tissue (MALT) may serve as a site for development of autoimmunity in other mucosal regions. Furthermore, in the previously discussed study by Fischer *et al.* [35], lung follicles consistent with iBALT were present in lung tissue from arthritis-free subjects with serum ACPAs suggesting this mechanism of autoantibody generation in RA may also be present in the preclinical period of RA.

What role do autoantibodies play in RA pathogenesis?

Much of our discussion above detailing the data that suggests the airways may be a potential initiating site of RA has focused on humoral immunity in the early steps of RA development. There are likely several reasons why the majority of these data have focused on humoral immune responses. These RA-related autoantibodies are readily obtainable and measurable in blood and other biospecimens. In particular, much of the data in preclinical RA utilized stored serum available in biospecimen repositories, and while prolonged sample storage does not significantly affect antibody testing, it may limit other immunologic testing [90]. As a result, little is known about the role of innate and cellular immunity during the preclinical period of RA development, and it remains unclear whether autoantibodies or other factors such as innate immunity and T cells are the true drivers of disease. However, supporting the importance of humoral immunity in the development of RA is established and emerging data that suggest that RA-related autoantibodies are indeed pathogenic in RA. In animal models, ACPAs have been shown to potentiate inflammatory arthritis [91], and ACPAs, specifically autoantibodies to citrullinated vimentin, have been shown to active osteoclasts [92]. Both ACPAs and RF are known to form immune complexes and activate macrophages [93,94]. Furthermore, the high specificity of RA-related autoantibodies, especially ACPAs, and studies showing rising levels of autoantibodies along with epitope spreading during preclinical RA, further support the likelihood that these autoantibodies are directly related to disease pathogenesis [14,17,95]. Alternatively, arguments against the direct pathogenicity of autoantibodies include the presence of autoantibodies in absence of synovitis in preclinical disease, the persistence of autoantibodies in many patients with RA even after resolution of synovitis [96], and the inability to readily induce RA with autoantibody transfer in animal models.

Because of these issues, going forward, a deeper understanding of the immune system beyond autoantibodies will be necessary to understand how RA may be initiated in the lung, or other mucosal surfaces, and in particular how an immune response generated at a mucosal site could transfer to another site, such as the

joints. Such studies should be facilitated by emerging technologies for studying T and B cells, as well as other aspects of the immune system (e.g., microparticles), in preclinical RA [97–99].

An overall model of the role of the lung in the pathogenesis of RA

If the lung is an initiating site of autoimmunity in the pathogenesis of RA, it may be through the model outlined in Figure 1. In this model, an inhaled environmental factor such as smoking or bacteria interacts with the host immune system at the surface of the airway mucosa. This interaction may trigger an inflammatory response that is initially localized to the lung in the form of iBALT, and in some individuals, this response may be an autoimmune response. Over time, however, this autoimmune response may transition to regional lymph nodes where it can then become systemic (i.e., the preclinical period of RA). After a period of circulating autoantibodies in preclinical RA, systemic autoimmunity may transition to clinically evident inflammatory arthritis that is classifiable as RA.

In this model, airways disease in RA may be the initial step, and lead to the generation of autoimmunity. Over time, inflammation- and autoimmune-mediated lung injury may occur through spread of inflammation from the airways to surrounding parenchymal structures. Or, perhaps epitope spreading within the lung or elsewhere in the body leads to targeting of lung-specific tissues. In particular, circulating autoimmunity may target parenchymal targets in the lung. It is not clear why the majority of patients who have lung disease in RA have minimally symptomatic disease, while some have fatal disease. The progression of lung disease in RA may be related to additional factors including environmental exposures such as smoking, infection and medication toxicity (or response to therapy leading to decreased lung disease), and autoimmune expansion to lung targets.

Ongoing challenges/unmet needs

Based on the data and concepts summarized herein, there is a growing appreciation that the lung plays an important role in the pathogenesis of RA. However, the specific role (e.g., initiation of inflammation and autoimmunity versus secondary target organ of autoantibodies or immune complexes) and the exact mechanisms including environmental triggers by which the lung is involved, in RA are unclear.

In order to understand these details, natural history studies of lung disease in RA are critical, including the natural history from preclinical RA to long-standing joint disease, specifically following the natural history of lung disease over that time. Such studies are needed to determine whether different types of lung disease in RA have a shared or unique etiopathogenesis as well as how these mechanisms of lung disease development relate to joint disease in RA, and in relationship to each other (e.g., airways disease potentially preceding parenchymal disease). As depicted in Figure 1, there may be multiple mechanisms of lung disease development after the initiation of autoimmunity in RA including systemic autoantibodies, immune complexes or inflammatory factors may target lung tissue, iBALT may result in local lung tissue damage, persistence of an inhaled antigen exposure may result in continued antigen-mediated immune reaction and inflammation at the airway mucosa, or additional environmental exposures may lead to progression of lung disease. It is also of interest that several other systemic connective tissue diseases can have significant lung manifestations, including some that can occur along with RA such as Sjogren's syndrome and systemic lupus erythematosus, and others that uncommonly overlap with RA such as systemic sclerosis, inflammatory myositis and the spondyloarthropathies [100]. Furthermore, it is recognized that scenarios exist whereby individuals have an 'autoimmune flavor' to their ILD (often limited to autoantibody positivity) but fall short of fulfilling existing diagnostic or classification criteria for any of the established connective tissue diseases [100,101]. Additional studies are needed to understand if these additional autoimmune-associated lung diseases are similar in pathogenesis to RA-related lung disease.

Furthermore, it remains difficult to predict clinically apparant lung disease onset and/or progression in RA due to an inadequacy of biomarkers specific for lung disease as well as variable interpretation of modalities such as CT imaging used to identify lung disease. Therefore, if biomarkers are identified that predict lung disease progression in RA, they will likely improve screening and/or treatment efforts of RA-related lung disease. Additionally, utilization of quantitative CT measures of lung disease may provide more standardized and comparable identification of lung disease in RA [102]. These issues are clinically significant given that it is currently difficult to council patients with symptomatic lung disease on prognosis, and the optimal treatment regimens for severe RA-related lung disease remains elusive. Management of patients with RA-related lung disease is a significant clinical challenge as there are no guidelines regarding screening, monitoring or treatment of symptomatic or asymptomatic lung disease in RA. It also remains difficult to identify asymptomatic subjects that would be important to enroll in natural history studies of RA-related lung diseases.

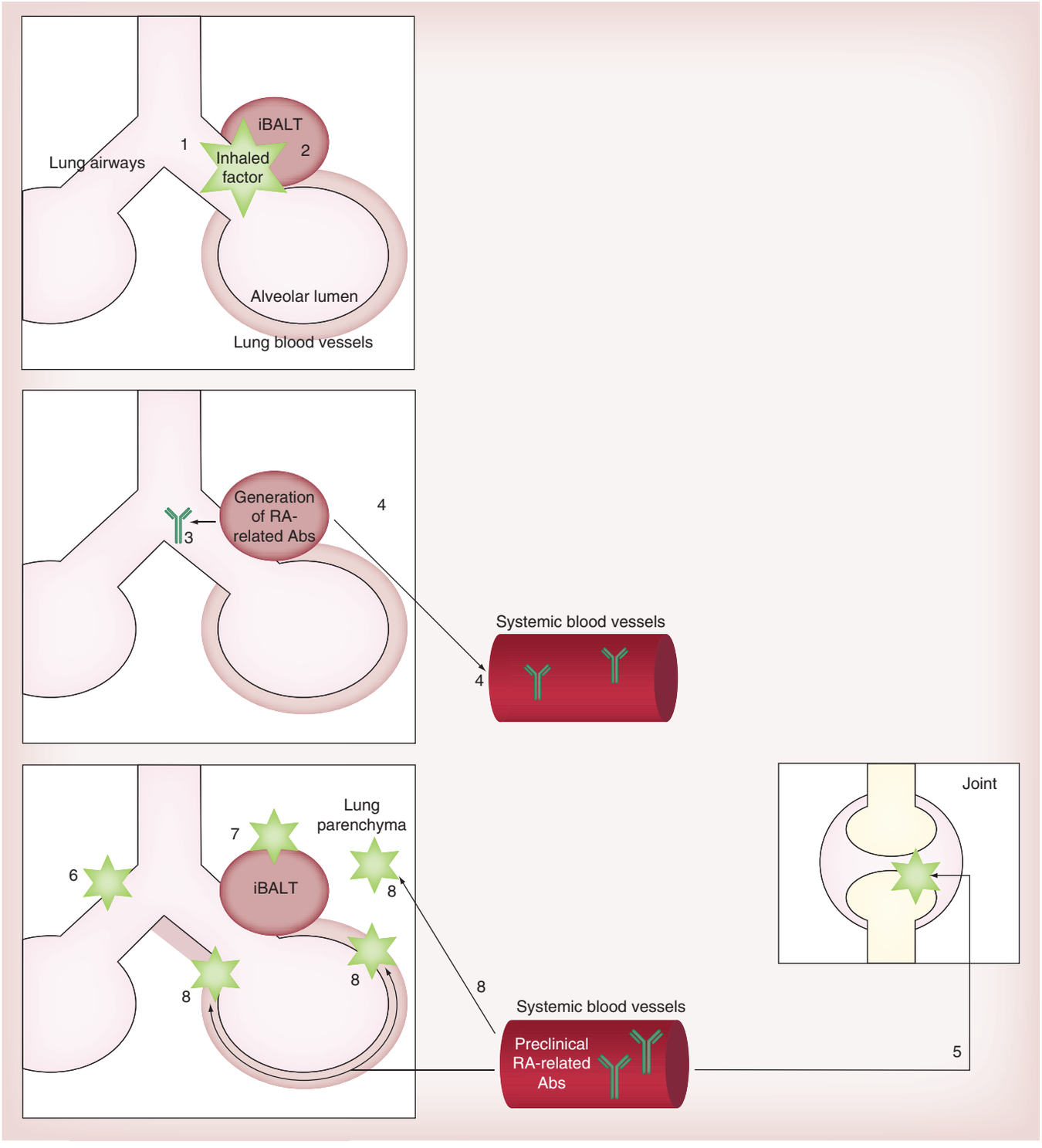

Figure 1. Model for the role of the lung in rheumatoid arthritis. A hypothetical model for the involvement of the lung in RA is depicted in which an inhaled environmental factor interacts with the host immune system at the airways mucosa **(1)**. This results in a local inflammatory immune reaction and induction of immune activity such as iBALT **(2)**. iBALT can result in local generation of autoantibodies in the lung **(3)**, but this can also transition to systemic autoimmunity through interactions with regional lymphatics **(4)**. Over time, systemic RA-related autoantibodies can transition to joint inflammation classifiable as RA through as-of-yet unknown mechanisms, but possibilities include circulating immune complexes depositing in the joints, or epitope spreading to include joint-specific antigens **(5)**. During the preclinical or later stages of RA, symptomatic or worsening lung disease may develop via persistent inhaled antigen exposure and ongoing airways inflammation that may also spread to the parenchyma **(6)**, iBALT may participate in

local lung tissue damage **(7)**, or circulating autoantibodies and other inflammatory factors may target the lung parenchyma **(8)**; other factors such as environmental exposures (e.g., smoking, infections, medications) may contribute to progressive lung disease.
Ab: Antibody; iBALT: Inducible bronchus associated lymphoid tissue; RA: Rheumatoid arthritis.

Another area of future study includes understanding the mechanisms by which autoimmunity can be initially generated in the lung, or perhaps other mucosal sites. In the study by Willis, Demoruelle and colleagues mentioned above, 39% of subjects without serum RA-related autoantibodies had at least one RA-related autoantibody present in sputum [53]. These results need to be repeated in additional studies; however, it is unlikely that many of these subjects will go on to develop RA or even systemic autoimmunity. However, could this high prevalence of RA-related autoantibodies in the lung be related to enhanced production of 'natural' autoantibodies at mucosal surfaces that serve as broad protectors against organisms or other environmental factors at the mucosal surface [103,104]? This will need to be explored in future studies. Furthermore, further investigations are needed to determine how autoimmunity could be initiated at a mucosal surface, and later transition to the joints. Several hypotheses have been proposed that include shared antigens between the lung or other mucosal surfaces, or epitope spreading from targets initially present at a mucosal surface to targets present in other tissues such as the joints, immune complex formation/deposition, and migration of activated T cells from the lung to the joint [94,105]. While IgA-mediated responses are not typically thought of as leading to immune complexes, in studies of juvenile arthritis, complement activating immune complexes with IgA–RF were detected in the serum and synovial fluid [106]. However, additional study is needed to understand if similar mechanisms are involved in RA, and in particular, comparative studies of mucosal surfaces and the joints need to be performed in order to understand the sequence of immunologic events in RA in these tissues. In addition, while RA is most commonly found in women, most studies of RA-related lung disease identify male sex as a risk factor for lung disease, yet the influence of sex hormones on the pathogenesis of RA-related lung disease, and especially how sex hormones may influence initiation of RA at a mucosal site, remains unknown. Interestingly on this front, a study by Keith and colleagues demonstrated that testosterone appeared to be protective against both lung disease and arthritis in a murine model of disease [107], although this is contrasted by studies in humans where men appear to be at higher risk for lung disease than women [30,108]. Finally, other mucosal sites may also generate RA-related autoimmunity, and similar investigations of other sites such as the gut, gingival and genitourinary mucosa are needed to understand the overall pathogenesis of RA.

Importantly, going forward the field will need to determine how best to use a variety of modalities to study the role of the lung as well as other mucosal sites in the natural history of RA. Current modalities include imaging and physiologic studies, as well as biospecimen collection and testing of samples such as exhaled breath condensates, BAL and sputum. In addition, there are growing numbers of animal models of RA-related lung disease that may prove informative [109]. Furthermore, the ability to assess biologic activity such as gene expression, and identify potential risk factors for disease such as microorganisms, is rapidly evolving. How all of these can be utilized in a safe and effective fashion to understand the role of the lung or other mucosal sites, as well as the development of joint disease, in human RA needs to be determined.

Future perspective

An emerging vision of the natural history of RA includes extra-articular sites such as the lung not only as tissue injured by autoimmune processes but as sites crucial to the initiation and propagation of autoimmunity. Over the next 5 years, this vision will lead to the development of strong natural history studies of RA in humans as well as informative animal models of disease that can leverage imaging, physiologic and biospecimen data, as well as advancing modalities to understand risk factors for disease (e.g., microbiome assessment) to understand the role of the lung in RA, and in particular the role that the lung may play in the initiation of disease. These studies will hopefully identify means to improve therapy for established disease, but ultimately may lead to methods that target the lung or other mucosal surfaces to prevent RA.

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Executive summary

Lung disease is highly prevalent in clinically classifiable rheumatoid arthritis

- Lung disease is prevalent in approximately 60–80% of patients with classifiable rheumatoid arthritis (RA); it is minimally symptomatic in most subjects, although for a minority, lung disease can be a severe manifestation of disease.

Differing risk factors & roles of airways & parenchymal lung disease in RA

- Airways and parenchymal lung disease may play different roles in the pathogenesis of RA.

Lung disease, & in particular airways disease, may occur prior to the onset of articular RA

- A high prevalence of airways disease during the preclinical period of RA strongly suggests that inflammation and autoimmunity in RA may be initiated at the airway mucosa, or targeted very early in disease development.

RA may originate at the airways mucosa

- Recent studies demonstrating RA-related autoantibodies in the sputum of subjects at risk for RA suggest autoimmunity in RA may be generated in the lung.

Mechanisms that may be involved in the generation of RA-related autoimmunity in the lung

- Factors that may trigger RA in the lung may include smoking or bacteria, but further studies are needed to determine how these factors can trigger autoimmunity.
- The mechanisms by which RA may be generated in the lung may include the development of inducible bronchus associated lymphoid tissue (iBALT) initiated following an inflammatory immune response to inhaled environmental factors.

Ongoing challenges/unmet needs

- Natural history studies of lung disease in RA are necessary to understand the different roles that the lung may play in the development and progression of RA.

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