*Commentary*

# Mucosa Biology and the Development of Rheumatoid Arthritis: Potential for Prevention by Targeting Mucosal Processes

M. Kristen Demoruelle, MD, PhD

*Division of Rheumatology, University of Colorado Denver, Aurora, CO, USA*

## ABSTRACT

As the goal in rheumatoid arthritis (RA) management shifts toward the prevention of joint disease, it is important to consider the role of mucosal sites in the pathogenesis of RA because they may be potential targets for preventive interventions. Multiple mucosal sites demonstrate immune dysregulation and inflammation in individuals with classifiable RA as well as, importantly, in individuals with systemic autoimmunity related to RA. The lung, gingival, and gastrointestinal mucosae are most strongly implicated in RA pathogenesis and may be sites where autoimmunity in RA initially develops. Targeting the exact site where the initial immune dysregulation in RA occurs is an appealing approach to prevention because it could avoid unwanted side effects of systemic therapies. However, several challenges must be addressed before mucosa-targeted interventions are a readily available option for RA prevention. Studies are needed to determine whether all RA-related immune dysregulation at mucosal sites will progress to joint disease and whether one or multiple mucosal sites demonstrate dysregulation prior to the development of classifiable RA. These areas of future research are likely to provide crucial pieces in the understanding of RA pathogenesis and ultimately RA prevention. (*Clin Ther*. 2019;41:1270–1278) © 2019 Published by Elsevier Inc.

**Key words:** autoimmunity, mucosal immunology, prevention, rheumatoid arthritis.

## INTRODUCTION

In the development of rheumatoid arthritis (RA), a phase of systemic autoimmunity associated with RA precedes the development of inflammatory arthritis by several years.[1–4] During this phase, systemic autoimmunity is present, but there are no signs of inflammatory arthritis, including no histologic evidence of synovitis.[5] The presence of systemic autoantibodies in the absence of joint inflammation supports that autoimmunity in RA originates at a site outside of the joints. Mucosal sites are a likely extraarticular site where RA-related autoimmunity originates because they can generate antigen-specific antibody responses to environmental factors, and as such, could aberrantly generate an autoimmune response to environmental factors. Herein, we will discuss the site-by-site basis for mucosal involvement in the development of RA.

## MUCOSAL HYPOTHESIS IN THE DEVELOPMENT OF RA

The identification of systemic autoimmunity preceding synovitis in RA has led to the hypothesis that the generation of RA-related autoimmunity originates at a mucosal surface. Because immunoglobulin (Ig)-A is the predominant antibody isotype of most mucosal sites, the mucosal hypothesis in the development of RA is further supported by a high prevalence of RA-related autoantibodies of the IgA isotype preceding RA, specifically anticitrullinated protein/peptide antibodies (ACPAs) and rheumatoid factor (RF).[1,6] In addition, the proportion of IgA plasmablasts is significantly greater than that of IgG plasmablasts in patients with systemic autoimmunity associated with RA, while healthy controls and patients with RA have a greater proportion of IgG compared to IgA plasmablasts.[7] Overall these data support the

*Accepted for publication April 8, 2019*
https://doi.org/10.1016/j.clinthera.2019.04.012
0149-2918/$ - see front matter

involvement of mucosal processes in the early generation of RA-related autoimmunity (Table I). However, it is unknown whether different mucosal sites in different individuals or multiple mucosal sites within an individual are involved in RA development.

It is important to consider that mucosal sites contain the mechanisms necessary to initiate immune responses, and therefore autoimmune responses. While different mucosal sites have some specialized immune features, all mucosal sites contain innate and adaptive immune effector cells, antibody-generating plasma cells, and inducible mucosa-associated lymphoid tissue that can generate antigen-specific antibodies.[25] In addition, an individual's immune system repeatedly interacts with environmental factors and the host microbiome at mucosal sites. These interactions are often localized and antiinflammatory, but in certain circumstances, a mucosal immune response to environmental factors or microbes can lead to systemic inflammation and antibodies.[26,27] As such, the inherent features of the mucosal immune system that are often protective against infection, have the ability to result in local and systemic antigen-specific autoimmune responses, as will be discussed.

## LINKS BETWEEN THE LUNG AND RA

It has long been established that there is a strong link between the lung and RA. Initial associations primarily focused on the lung as an extraarticular organ affected by RA pathogenesis in individuals with established RA.[28,29] However, more recent data have made clear that the lung also likely contributes to the development of RA, particularly the development of ACPA-positive RA.[8,9]

Lung involvement has been identified during all phases of RA development, from early to chronic RA and even during pre-RA and systemic autoimmunity associated with RA.[9,29–31] All anatomic sites of the lung can be affected in RA, including the parenchyma, airway, pleura, and vasculature of the lung.[28] Depending on the modality used for screening, over 60% of patients with established RA demonstrate some underlying lung abnormality.[29,32] Although only a small percentage of those abnormalities are symptomatic from lung disease (10%–20%), it is clear that the lung is an organ commonly involved in RA.

Studies of environmental risk factors in RA have led the way to considering the lung as a major contributing factor in the development of RA, rather than as simply a potential target of autoimmunity. In particular, cigarette smoking has long been a strong risk factor for developing RA.[33] Importantly, this risk is dose dependent and remains elevated for many years after smoking cessation.[34–36] Other inhalational exposures such as silica dust and air pollution have also been

Table I. Data supporting associations between mucosal sites and rheumatoid arthritis (RA).

| Mucosal Site | Data Supporting Association With RA |
|---|---|
| Lung mucosa | Strong association between smoking and ACPA + RA[8]; increased prevalence of airway inflammation in systemic autoimmunity associated with RA[9]; ACPA and RF generation in the lungs of patients with RA, patients with systemic autoimmunity associated with RA, and patients with genetic risk factors for RA[10–12] |
| Gingival mucosa | Periodontitis prevalence is increased in patients with RA[13,14]; *Porphyromonas gingivalis* contains an enzyme that can citrullinate proteins,[15] and antibodies to *P gingivalis* have been associated with RA[16–18]; *Aggregatibacter actinomycetemcomitans* leukotoxin induces citrullinated proteins in neutrophils that are similar to citrullinated proteins in RA synovium, and antibodies to this leukotoxin are increased in RA[19] |
| Gastrointestinal mucosa | RA has been associated with expansions and reductions in stool microbiota[20–22]; the relative abundance of *Prevotella copri* is expanded in the stools of patients with new-onset RA,[23] and antibody responses to *P copri* are increased in RA[24] |

ACPA = anticitrullinated protein/peptide antibody.

associated with an increased RA risk,[37,38] suggesting that it is the shared lung exposure associated with RA risk more so than specific components of cigarette smoke. Further support for the relationship between the development of RA and the lung came from a 2006 study by Klareskog et al,[8] in which a 21-fold increased risk for ACPA-positive RA was found in individuals with human leukocyte antigen—DR isotype shared epitope genetic risk alleles and a history of cigarette smoking. Taking this data into account along with data from prior studies that demonstrated increased citrullinated proteins in the lungs of cigarette smokers and ACPA generation in inducible bronchus-associated lymphoid tissue in the lungs of patients with RA,[10,39] these data in aggregate support a hypothesis that smoking can lead to ACPA-positive RA via the generation of citrullinated proteins and ACPA in the lung. However, additional studies are needed to confirm that inflammation and ACPA generation do in fact occur in the lung in the early steps of RA development.

One of the first studies to identify lung inflammation in individuals with systemic autoimmunity associated with RA was a 2013 study by our group that identified a significantly greater prevalence of airway abnormalities on high-resolution computed tomography compared to that in serum antibody—negative controls (76% vs 33%; $P < 0.01$).[9] The specific airway abnormalities identified in association with serum ACPA and RF primarily involved the small airways of the lung (eg, bronchial wall thickening and air trapping), supporting that these findings could have been the result of inflammation associated with an inhaled exposure. Our group went on to use induced sputum, which is a biospecimen collected from a deep cough that samples the airways of the lung, to confirm that ACPA and RF can be generated locally in the lung in patients with RA, patients with systemic autoimmunity associated with RA, and patients with genetic risk factors for RA.[11,12,40] However, these antibodies can also be generated in association with non-RA chronic inflammatory lung disease, and citrulline specificity of lung antibodies appears to be a feature more strongly associated with classified RA.[41,42] Importantly, these data have established the lung as a site of RA-related autoantibody generation, even in RA-free patients with risk factors for RA, supporting the hypothesis that the lung contributes to the development of RA.

In addition to the generation of RA-related autoantibodies, the lung may play a role in propagating autoimmunity in RA. Epitope spreading is an established phenomenon that occurs systemically during pre-RA,[43] resulting in ACPAs that target an increasing number of citrullinated epitopes. A recent study from our group that investigated individual citrullinated protein/peptide reactivities in sputum found that a similar phenomenon may occur in the lung as well,[12] although longitudinal studies are needed to confirm this hypothesis.

Other factors that influence the mucosal immune system include interactions with the mucosal microbiome. Microbes are integral to the development of a robust immune system, but alterations in microbial communities can lead to dysregulated immune responses and have been associated with autoimmune disease.[44] In contrast to the gingival and gut microbiomes, which will be discussed later, there is a limited understanding of how the lung microbiome influences the development of RA. A study by Scher et al[45] investigated the lung microbiome in individuals with early, untreated RA using bronchoalveolar lavage fluid. That study found decreased diversity of the distal lung microbiome in patients with RA compared to healthy controls with several bacteria associated with RA disease activity, and systemic and bronchoalveolar lavage fluid ACPA levels. Going forward, it will be important to understand microbial alterations in the lung preceding arthritis onset, as microbiome changes later in the development of RA could be the result of systemic inflammation rather than causal in disease pathogenesis. Ultimately, understanding the complex interplay between inhaled exposures, inflammation, microbes, and autoimmunity in RA will be crucial to fully understand the role of the lung microbiome in the development of RA.

## THE GINGIVAL MUCOSA AND RA

The gingival mucosa is another mucosal site that has been strongly linked to RA for many years. Initial associations were primarily epidemiologic, with the now—well-established finding of an increased prevalence of periodontal disease in individuals with established RA,[13,14] particularly ACPA-positive RA. Periodontitis has also been associated with greater levels of citrullinated proteins and RA-related

autoantibodies.[19,46] It is of interest that RA and periodontitis also share risk factors of smoking, obesity, and genetic risk alleles.[47] A full understanding of these relationships has been complicated by the cross-sectional nature of most studies and variability in the criteria used to define periodontitis.

Similar to the lung mucosa, ACPA generation has been demonstrated in the gingival mucosa associated with inflammation in individuals without RA.[48] However, in contrast to the lung, the gingival mucosa is strongly linked to RA through associations with specific bacteria, particularly the periodontitis-associated *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*.[16,17]

A pathogenic link to RA has been speculated for *P gingivalis* through its unique expression of a bacterial peptidylarginine deiminase enzyme that can citrullinate proteins, which can lead to targets of ACPA generated in the gingival mucosa.[15] In addition, individuals with RA and systemic autoimmunity related to RA often demonstrate greater serum levels of antibodies to *P gingivalis*,[16–18] and *P gingivalis* can induce and exacerbate arthritis in animal models.[49,50] A different pathogenic mechanism has been hypothesized for the link between *A actinomycetemcomitans* and RA. A leukotoxin produced by *A actinomycetemcomitans* can disrupt the cellular membrane of neutrophils, leading to hypercitrullination,[19] and the citrullinated proteins formed during this process are of a pattern similar to that found in RA synovium.[19] Individuals with RA also had increased levels of antibodies to the *A actinomycetemcomitans*–associated leukotoxin that were associated with certain ACPA reactivities.

Overall, these data suggest a connection between RA and specific bacteria in the gingiva. However, periodontitis has also been associated with increased citrullinated proteins independent of *P gingivalis* and *A actinomycetemcomitans*.[46] Moreover, in patients with RA without periodontitis, the subgingival microbiome differed compared to that in controls, and it was *Cryptobacterium curtum* that was associated with RA.[20,51] *C curtum* is a bacterium known to produce citrulline, raising the likelihood that the function of these bacteria, rather than their molecular structure, may contribute to RA pathogenesis.

## DYSBIOSIS IN THE GASTROINTESTINAL MUCOSA IN RA

The gastrointestinal mucosa has a major impact on the development and maintenance of an individual's immune system, particularly through interactions with the large volume of microbiota lining the gut mucosa.[52] These features support that the gastrointestinal mucosa could play a role in RA pathogenesis. However, studies supporting an association between the gut and RA pathogenesis are most often limited to individuals who have already developed RA, making it difficult to determine whether these findings are causal in the development of RA.

Similar to the gingival mucosa, specific bacteria in the gastrointestinal tract have been implicated in ACPA and RA pathogenesis, most notably *Prevotella copri*. It was first reported by Scher et al[23] that the relative abundance of *P copri* was expanded in the stool of patients with new-onset RA compared to controls. Subsequently, it has been demonstrated that antibody responses to *P copri* are increased in patients with RA compared to controls,[24] and *P copri*–derived peptides can activate T cells in patients with RA. Recently, *Prevotella* was found to be increased in the stool of patients with systemic autoimmunity associated with RA.[53]

However, expansions as well as reductions of other gut bacteria have also been associated with RA,[20–22] and animal models of arthritis, including the collagen-induced arthritis model, demonstrate associations between gut dysbiosis and arthritis that are not dependent on *P copri*.[54,55] It may be that, similar to the gingival mucosa, it is the function of mucosal bacteria and not their molecular structure that contributes to RA pathogenesis.

## ADDITIONAL CONSIDERATIONS IN MUCOSA-BASED PREVENTION STRATEGIES FOR RA

The lung, gingival, and gastrointestinal mucosae are the most well studied in RA, but the nasopharyngeal and urogenital tract mucosae should not be disregarded. Similar to the other mucosal sites discussed, they can generate antigen-specific antibody responses and regularly encounter various environmental factors. Prior data have identified elevations in serum antibodies to urinary *Proteus mirabilis* in patients with RA compared to those in

controls.[56] Regarding the cervicovaginal mucosa in women, our group has identified the generation of ACPA in the cervicovaginal mucosa of women with and without RA.[57] The cervicovaginal mucosa is of particular interest in the development of RA given that it is unique to women, is influenced by sex hormones, and has the potential to improve the understanding of why women develop RA more often than men. More studies are needed going forward to understand the contribution of these mucosal sites in the development of RA.

## POTENTIAL MUCOSA-BASED STRATEGIES OF RA PREVENTION

With the strength of associations between mucosal sites and RA as described here, particularly the associations during systemic autoimmunity associated with RA, it is likely that mucosal sites must be considered in the prevention of RA. These mucosa-based approaches could be primary or complementary to other preventive interventions. It is unknown which strategies of RA prevention will be most effective, but approaches such as smoking cessation, dental hygiene, and diet may have their effects through mucosa-based immune regulation. In addition, future prevention studies should consider therapies that are delivered directly to mucosal sites, such as inhaled, topical, or oral therapies. It may be that personalized approaches are needed to be effective in the prevention of RA, and personalization based on the mucosal site demonstrating an RA-related dysregulated immune response within an individual may be a novel and effective approach.

## CHALLENGES IN MUCOSA-BASED RA PREVENTION

Although it is appealing to employ mucosa-based approaches to RA prevention, there remain several

Table II. Major challenges in the area of rheumatoid arthritis (RA)-related mucosal biology.

| Challenge | Questions | Needs |
|---|---|---|
| Identifying when in the course of RA development mucosal sites play a role | Are mucosal sites involved in triggering and/or propagating autoimmunity? Do they also contribute to transitions from systemic autoimmunity to inflammatory arthritis? | Longitudinal natural history studies of individuals with risk factors for RA |
| Understanding how the microbiome at a mucosal surface influences RA development | Do microbes influence RA develop through direct effects (eg, molecular mimicry or shaping the immune system) or indirect effects (eg, enzymatic or metabolomic consequences of microbes or bystander activation from microbe-induced inflammation)? | Microbiome studies that address both direct and indirect effects of microbes |
| Determining whether mucosal sites are independent or synergistic in the generation or propagation of RA-related autoimmunity | In a given individual who ultimately develops RA, is a single mucosal site involved, but the specific site could differ between individuals? or are multiple mucosal sites involved within an individual and a synergistic or cumulative effect occurs? | Studies that simultaneously sample multiple mucosal sites in individuals with systemic autoimmunity related to RA |
| Integrating the multiple factors that could influence RA-related autoimmunity at mucosal surfaces. | Do environmental factors (eg, smoking) trigger and propagate autoimmunity in the lung independently, or do they require 'co-factors' such as microbial factors and/or genetic factors to have an influence on the development of RA? | Analytic approaches that can appropriately take into account the multiple factors influencing mucosal immune responses and autoimmunity |

challenges (Table II). It remains unclear when in the course of RA development mucosal factors play a role. Do they contribute to the initial generation and/or propagation of autoimmunity in pre-RA, contribute to transitions from systemic autoimmunity to inflammatory arthritis, or both? If an autoimmune response originates at a mucosal site, such as the lung, how does it ultimately lead to inflammatory arthritis? Hypotheses include protein targets shared between the 2 sites, molecular mimicry between mucosal microbiota and joint proteins, activation of autoreactive T cells through inflammation, and bystander activation and formation of immune complexes that deposit in the joint.[58,59] An improved understanding of these transitions between mucosal, systemic, and synovial processes will likely be needed to effectively target mucosal sites in the prevention of RA.

Another important consideration in mucosal site involvement in RA development is whether mucosal sites are independent or synergistic with one another in the generation or propagation of RA-related autoimmunity. Zhang et al[20] compared the microbiomes of the gut and oral mucosa in patients with RA and found similar changes at each site, with a relative depletion of gram-negative bacteria and enrichment of gram-positive bacteria. Also of interest, Bradley et al[60] found that a particular bacterium acting in the gastrointestinal mucosa can influence inflammation, T-cell recruitment, and antibody generation in the lung during a prearthritis phase in animal models. Future studies are clearly needed to better understand the relationship between different mucosal sites during systemic autoimmunity associated with RA. In addition, these data raise questions of exactly how the microbiome may contribute to RA development. Hypotheses include *molecular mimicry*, in which an immune response to microbial epitopes leads to a break in tolerance to self-antigens (eg, rheumatic fever); general shaping of the immune system by microbiota that promotes the development of autoreactive cells; production of citrullinated proteins or neoantigens through enzymatic or metabolomic effects of microbes; and microbial communities that lead to an inflammatory environment and could provide inflammatory cofactors (eg, second messengers) to activate autoreactive cells.

## CONCLUSIONS

Multiple mucosal sites, particularly the lung, gingival, and gut mucosae, likely play a key role in the development of RA and RA-related autoimmunity. As such, mucosal sites must be considered in approaches to RA prevention. However, an improved understanding of all mucosal sites and their interactions with each other is needed to most effectively utilize mucosa-based approaches to RA prevention.

## CONFLICTS OF INTEREST

The author has indicated that she has no conflicts of interest with regard to the content of this article.

## ACKNOWLEDGEMENT

I have received research grants from the NIH AR066712, HD057022, AI101981 and the Rheumatology Research Foundation, although they are not directly involved in this manuscript. The author contributed to the literature search, data interpretation and writing of the manuscript.

## REFERENCES

1. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum*. 2003;48:2741–2749.
2. Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum*. 2004;50:380–386.
3. Majka DS, Deane KD, Parrish LA, et al. Duration of preclinical rheumatoid arthritis-related autoantibody positivity increases in subjects with older age at time of disease diagnosis. *Ann Rheum Dis*. 2008;67:801–807.
4. Gerlag DM, Raza K, van Baarsen LG, et al. EULAR recommendations for terminology and research in individuals at risk of rheumatoid arthritis: report from the Study Group for Risk Factors for Rheumatoid Arthritis. *Ann Rheum Dis*. 2012;71:638–641.
5. van de Sande MG, de Hair MJ, van der Leij C, et al. Different stages of rheumatoid arthritis: features of the synovium in the preclinical phase. *Ann Rheum Dis*. 2011;70:772–777.
6. Kokkonen H, Mullazehi M, Berglin E, et al. Antibodies of IgG, IgA and IgM isotypes against cyclic citrullinated peptide precede the development of rheumatoid arthritis. *Arthritis Res Ther*. 2011;13:R13.

7. Kinslow JD, Blum LK, Deane KD, et al. Elevated IgA Plasmablast levels in subjects at risk of developing rheumatoid arthritis. *Arthritis Rheumatol*. 2016;68:2372–2383.
8. Klareskog L, Stolt P, Lundberg K, et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. *Arthritis Rheum*. 2006;54:38–46.
9. Demoruelle MK, Weisman MH, Simonian PL, et al. Brief report: airways abnormalities and rheumatoid arthritis-related autoantibodies in subjects without arthritis: early injury or initiating site of autoimmunity? *Arthritis Rheum*. 2012;64:1756–1761.
10. Rangel-Moreno J, Hartson L, Navarro C, Gaxiola M, Selman M, Randall TD. Inducible bronchus-associated lymphoid tissue (iBALT) in patients with pulmonary complications of rheumatoid arthritis. *J Clin Invest*. 2006;116:3183–3194.
11. Demoruelle MK, Harrall KK, Ho L, et al. Anti-citrullinated protein antibodies are associated with neutrophil extracellular traps in the sputum in relatives of rheumatoid arthritis patients. *Arthritis Rheumatol*. 2017;69:1165–1175.
12. Demoruelle MK, Bowers E, Lahey LJ, et al. Antibody responses to citrullinated and noncitrullinated antigens in the sputum of subjects with rheumatoid arthritis and subjects at risk for development of rheumatoid arthritis. *Arthritis Rheumatol*. 2018;70:516–527.
13. Fuggle NR, Smith TO, Kaul A, Sofat N. Hand to Mouth: a systematic review and meta-analysis of the association between rheumatoid arthritis and periodontitis. *Front Immunol*. 2016;7:80.
14. Mikuls TR, Payne JB, Yu F, et al. Periodontitis and *Porphyromonas gingivalis* in patients with rheumatoid arthritis. *Arthritis Rheumatol*. 2014;66:1090–1100.
15. Wegner N, Wait R, Sroka A, et al. Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and alpha-enolase: implications for autoimmunity in rheumatoid arthritis. *Arthritis Rheum*. 2010;62:2662–2672.
16. Mikuls TR, Thiele GM, Deane KD, et al. Porphyromonas gingivalis and disease-related autoantibodies in individuals at increased risk of rheumatoid arthritis. *Arthritis Rheum*. 2012;64:3522–3530.
17. Hitchon CA, Chandad F, Ferucci ED, et al. Antibodies to *Porphyromonas gingivalis* are associated with anticitrullinated protein antibodies in patients with rheumatoid arthritis and their relatives. *J Rheumatol*. 2010;37:1105–1112.
18. Kharlamova N, Jiang X, Sherina N, et al. Antibodies to *Porphyromonas gingivalis* indicate interaction between oral infection, smoking, and risk genes in rheumatoid arthritis etiology. *Arthritis Rheumatol*. 2016;68:604–613.
19. Konig MF, Abusleme L, Reinholdt J, et al. *Aggregatibacter actinomycetemcomitans*-induced hypercitrullination links periodontal infection to autoimmunity in rheumatoid arthritis. *Sci Transl Med*. 2016;8, 369ra176.
20. Zhang X, Zhang D, Jia H, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat Med*. 2015;21:895–905.
21. Vaahtovuo J, Munukka E, Korkeamaki M, Luukkainen R, Toivanen P. Fecal microbiota in early rheumatoid arthritis. *J Rheumatol*. 2008;35:1500–1505.
22. Liu X, Zou Q, Zeng B, Fang Y, Wei H. Analysis of fecal *Lactobacillus* community structure in patients with early rheumatoid arthritis. *Curr Microbiol*. 2013;67:170–176.
23. Scher JU, Sczesnak A, Longman RS, et al. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *Elife*. 2013;2, e01202.
24. Pianta A, Arvikar S, Strle K, et al. Evidence of the Immune Relevance of *Prevotella copri*, a gut microbe, in patients with rheumatoid arthritis. *Arthritis Rheumatol*. 2017;69:964–975.
25. Janeway Jr CA, Travers P, Walport M, Shlomchik MJ. *Immunobiology: The Immune System in Health and Disease*. 5th ed. New York, NY: Garland Science; 2001.
26. Chu H, Khosravi A, Kusumawardhani IP, et al. Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. *Science*. 2016;352:1116–1120.
27. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science*. 2012;336:1268–1273.
28. Yunt ZX, Solomon JJ. Lung disease in rheumatoid arthritis. *Rheum Dis Clin North Am*. 2015;41:225–236.
29. Gabbay E, Tarala R, Will R, et al. Interstitial lung disease in recent onset rheumatoid arthritis. *Am J Respir Crit Care Med*. 1997;156(2 Pt 1):528–535.
30. Reynisdottir G, Olsen H, Joshua V, et al. Signs of immune activation and local inflammation are present in the bronchial tissue of patients with untreated early rheumatoid arthritis. *Ann Rheum Dis*. 2016;75:1722–1727.
31. Fischer A, Solomon JJ, du Bois RM, et al. Lung disease with anti-CCP antibodies but not rheumatoid arthritis or connective tissue disease. *Respir Med*. 2012;106:1040–1047.
32. Reynisdottir G, Karimi R, Joshua V, et al. Structural changes and antibody enrichment in the lungs are early features of anti-citrullinated protein antibody-positive rheumatoid arthritis. *Arthritis Rheumatol*. 2014;66:31–39.

33. Silman AJ, Newman J, MacGregor AJ. Cigarette smoking increases the risk of rheumatoid arthritis. Results from a nationwide study of disease-discordant twins. *Arthritis Rheum*. 1996;39:732–735.
34. Di Giuseppe D, Discacciati A, Orsini N, Wolk A. Cigarette smoking and risk of rheumatoid arthritis: a dose-response meta-analysis. *Arthritis Res Ther*. 2014;16:R61.
35. Costenbader KH, Feskanich D, Mandl LA, Karlson EW. Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women. *Am J Med*. 2006;119:503 e1–9.
36. Liu X, Tedeschi SK, Barbhaiya M, et al. Impact and timing of smoking cessation on reducing risk for rheumatoid arthritis among women in the Nurses' Health Studies. *Arthritis Care Res (Hoboken)*. 2019 Feb 21 [Epub ahead of print].
37. Stolt P, Kallberg H, Lundberg I, et al. Silica exposure is associated with increased risk of developing rheumatoid arthritis: results from the Swedish EIRA study. *Ann Rheum Dis*. 2005;64:582–586.
38. Hart JE, Kallberg H, Laden F, et al. Ambient air pollution exposures and risk of rheumatoid arthritis: results from the Swedish EIRA case-control study. *Ann Rheum Dis*. 2013;72:888–894.
39. Makrygiannakis D, Hermansson M, Ulfgren AK, et al. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. *Ann Rheum Dis*. 2008;67:1488–1492.
40. Willis VC, Demoruelle MK, Derber LA, et al. Sputum autoantibodies in patients with established rheumatoid arthritis and subjects at risk of future clinically apparent disease. *Arthritis Rheum*. 2013;65:2545–2554.
41. Quirke AM, Perry E, Cartwright A, et al. Bronchiectasis is a model for chronic bacterial infection inducing autoimmunity in rheumatoid arthritis. *Arthritis Rheumatol*. 2015;67:2335–2342.
42. Janssen KM, de Smit MJ, Brouwer E, et al. Rheumatoid arthritis-associated autoantibodies in non-rheumatoid arthritis patients with mucosal inflammation: a case-control study. *Arthritis Res Ther*. 2015;17:174.
43. Sokolove J, Bromberg R, Deane KD, et al. Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. *PLoS One*. 2012;7: e35296.
44. Lerner A, Aminov R, Matthias T. Dysbiosis may trigger autoimmune diseases via inappropriate post-translational modification of host proteins. *Front Microbiol*. 2016;7:84.
45. Scher JU, Joshua V, Artacho A, et al. The lung microbiota in early rheumatoid arthritis and autoimmunity. *Microbiome*. 2016;4:60.
46. Engstrom M, Eriksson K, Lee L, et al. Increased citrullination and expression of peptidylarginine deiminases independently of *P. gingivalis* and *A. actinomycetemcomitans* in gingival tissue of patients with periodontitis. *J Transl Med*. 2018;16:214.
47. Stabholz A, Shapira L, Mahler D, et al. Using the PerioChip in treating adult periodontitis: an interim report. *Compend Contin Educ Dent*. 2000;21:325–328, 30, 32 passim; quiz 38.
48. Harvey GP, Fitzsimmons TR, Dhamarpatni AA, Marchant C, Haynes DR, Bartold PM. Expression of peptidylarginine deiminase-2 and -4, citrullinated proteins and anti-citrullinated protein antibodies in human gingiva. *J Periodontal Res*. 2013;48:252–261.
49. Courbon G, Rinaudo-Gaujous M, Blasco-Baque V, et al. *Porphyromonas gingivalis* experimentally induces periodontis and an anti-CCP2-associated arthritis in the rat. *Ann Rheum Dis*. 2019;78:594–599.
50. Marchesan JT, Gerow EA, Schaff R, et al. *Porphyromonas gingivalis* oral infection exacerbates the development and severity of collagen-induced arthritis. *Arthritis Res Ther*. 2013;15:R186.
51. Lopez-Oliva I, Paropkari AD, Saraswat S, et al. Dysbiotic subgingival microbial communities in periodontally healthy patients with rheumatoid arthritis. *Arthritis Rheumatol*. 2018;70:1008–1013.
52. Longman RS, Littman DR. The functional impact of the intestinal microbiome on mucosal immunity and systemic autoimmunity. *Curr Opin Rheumatol*. 2015;27:381–387.
53. Alpizar-Rodriguez D, Lesker TR, Gronow A, et al. *Prevotella copri* in individuals at risk for rheumatoid arthritis. *Ann Rheum Dis*. 2019;78:590–593.
54. Liu X, Zeng B, Zhang J, et al. Role of the gut microbiome in modulating arthritis progression in mice. *Sci Rep*. 2016;6:30594.
55. Jubair WK, Hendrickson JD, Severs EL, et al. Modulation of inflammatory arthritis in mice by gut microbiota through mucosal inflammation and autoantibody generation. *Arthritis Rheumatol*. 2018;70:1220–1233.
56. Wilson C, Thakore A, Isenberg D, Ebringer A. Correlation between anti-*Proteus* antibodies and isolation rates of *P. mirabilis* in rheumatoid arthritis. *Rheumatol Int*. 1997;16:187–189.
57. Khatter S, Berens-Norman H, Anderson C, et al. Anti-CCP antibody levels are elevated in cervicovaginal fluid in association with local inflammation in premenopausal women without RA [abstract]. *Arthritis Rheumatol*. 2017;69(suppl 10):4205–4206.
58. Ytterberg AJ, Joshua V, Reynisdottir G, et al. Shared immunological targets in the lungs and joints of patients with rheumatoid arthritis: identification

and validation. *Ann Rheum Dis*. 2015;74:1772–1777.
59. Li S, Yu Y, Yue Y, et al. Autoantibodies from single circulating plasmablasts react with citrullinated antigens and *Porphyromonas gingivalis* in rheumatoid arthritis. *Arthritis Rheumatol*. 2016;68:614–626.
60. Bradley CP, Teng F, Felix KM, et al. Segmented filamentous bacteria provoke lung autoimmunity by inducing gut-lung Axis Th17 cells expressing dual TCRs. *Cell Host Microbe*. 2017;22:697–704 e4.

**Address correspondence to:** M. Kristen Demoruelle, MD, PhD, 1775 Aurora Court, Mail Stop B-115, Aurora, CO 80045, USA. E-mail: kristen.demoruelle@ucdenver.edu