

Autoimmunity of the lung and oral mucosa in a multisystem inflammatory disease: The spark that lights the fire in rheumatoid arthritis?



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There is a growing body of evidence to suggest that autoimmunity in patients with rheumatoid arthritis (RA) is initiated outside the joint. This is supported by the observation that circulating autoantibodies, including both rheumatoid factor and anti-citrullinated protein antibody, can be detected in many subjects years before the development of initial joint symptoms leading to an RA diagnosis. Of the potential extra-articular sites implicated in disease initiation, mucosal tissues have garnered increasing attention. Several lines of investigation have separately implicated mucosal tissues from varying anatomic locations as possible initiating sites for RA, including those from the lung and oral cavity. In this review we summarize recent reports incriminating these mucosal tissues as the initial site of autoantibody generation and inflammation in patients with RA. (*J Allergy Clin Immunol* 2016;137:28-34.)

Key words: Rheumatoid arthritis, autoantibody, mucosal inflammation, anti-citrullinated protein antibody, rheumatoid factor

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Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown cause, with its primary manifestation in synovial joints. Affecting up to 1% of the population, RA is associated with substantial morbidity, disability, reduced survival, and substantial and growing health care costs.¹

There have been substantial gains made in our understanding of RA pathogenesis, including the prominent (and in some cases pathogenic) role that autoantibodies play in disease onset and progression. Described more than 50 years ago,² rheumatoid factor (RF) is an antibody that binds to the Fc portion of IgG and is detected in 70% to 80% of patients with RA. More recent discoveries have focused on anti-citrullinated protein antibodies (ACPAs), autoantibodies that, in contrast to RF, are highly

Abbreviations used

ACPA:	Anti-citrullinated protein antibody
BAL:	Bronchoalveolar lavage
CarP:	Carbamylated protein
CCP:	Cyclic citrullinated peptide
HRCT:	High-resolution computed tomography
MAA:	Malondialdehyde-acetaldehyde
PAD:	Peptidylarginine deiminase
PD:	Periodontitis
RA:	Rheumatoid arthritis
RF:	Rheumatoid factor

disease specific (typically >90% specific³) and bind to peptides that have undergone posttranslational modifications facilitated by a family of peptidylarginine deiminases (PADs).^{4,5} Recent data have suggested that the PAD4 isoenzyme might itself be immunogenic in patients with RA with anti-PAD4 antibody driving disease-related inflammation and damage.⁴ Other more recently described disease-related autoantibodies also target peptides that have undergone posttranslational change, including antibodies to carbamylated proteins (CarPs) and malondialdehyde-acetaldehyde (MAA; Fig 1).^{6,7} In contrast to the process of citrullination, the formation of both CarPs and MAA are not enzymatically catalyzed. CarPs are produced when cyanate reacts with the primary amine on lysine residues, forming homocitrulline. Also formed primarily on lysine residues, MAA adducts are produced as a result of oxidative stress and lipid peroxidation.^{8,9} It is noteworthy that all of these posttranslational changes, including citrullination, MAA adduct formation, and carbamylation, might be facilitated by innate immune responses, namely neutrophil-driven responses serving as a possible link between innate immunity and autoimmunity.¹⁰

Although RA as a clinical disease is primarily classified based on the presence of articular inflammation, there is a growing body of evidence to suggest that RA autoimmunity can be initiated in extra-articular compartments. This is supported by the observation that circulating autoantibodies (RF, ACPA, anti-PAD4, and anti-CarP) can be detected years before patients have joint symptoms¹¹⁻¹³ and in some cases in the absence of demonstrable arthritis based on physical examination, imaging, and even synovial biopsy.^{14,15} Of the potential extra-articular sites implicated in disease initiation, mucosal tissues have garnered increasing attention. Specifically, several lines of investigation have implicated mucosal tissues from the lung and oral cavity, with separate lines of evidence pointing toward the gastrointestinal and genitourinary tracts as possible initiating sites for RA.

In this review we summarize recent human studies incriminating mucosal tissues of the lung or oral cavity as the initial site of autoantibody generation and inflammation in patients with RA.

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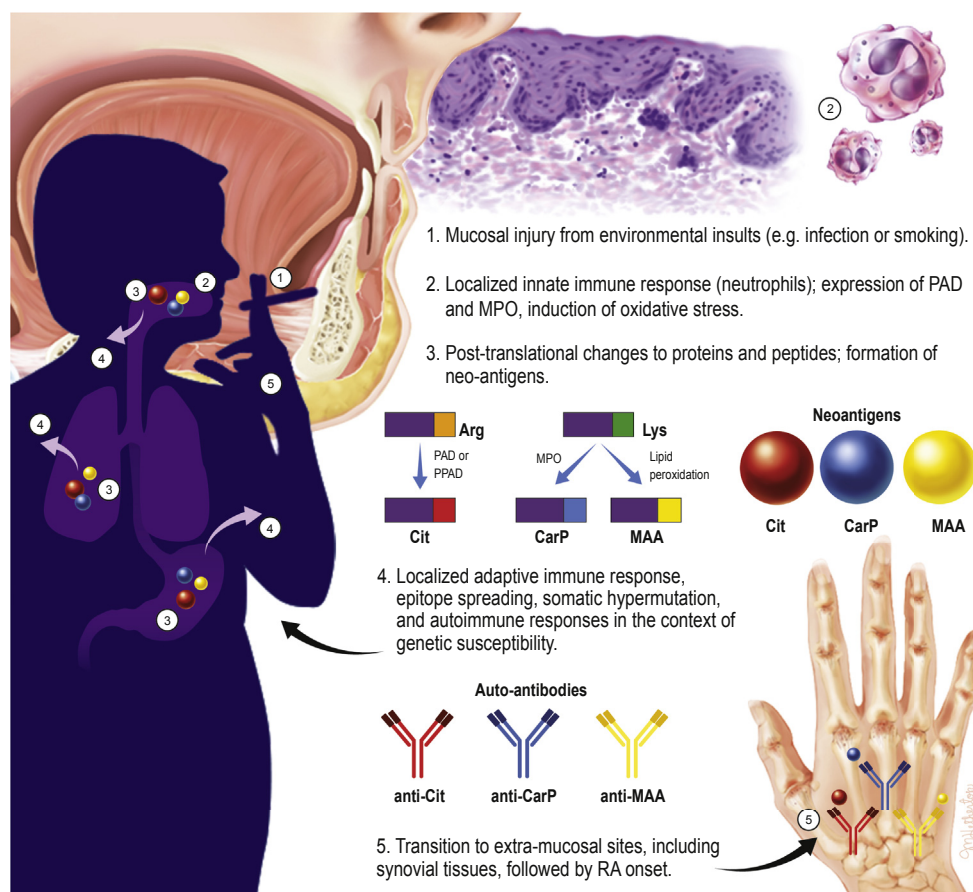


FIG 1. Proposed mechanisms linking chronic mucosal inflammation with the development of RA. 1, Environmental triggers, such as cigarette smoking or chronic infection (eg, periodontitis), result in localized innate immune responses (eg, gingivitis, periodontitis, and bronchitis) driven primarily by neutrophils. 2, Innate immune responses lead to the generation and/or increased expression of PAD and myeloperoxidase (MPO) and lead to increased oxidative stress and lipid peroxidation. 3, These factors promote posttranslational protein/peptide modifications and neoantigen formation. *Arg*, Arginine; *Lys*, lysine; *PPAD*, *P gingivalis*-expressed PAD. 4, After epitope spreading and somatic hypermutation, disease-related autoantibodies are expressed, including ACPAs and antibodies to MAA and CarP. 5, Autoimmunity that begins in mucosal tissues in the lung or oral cavity transitions to extramucosal sites, eventually leading to the signs and symptoms characteristic of RA.

MUCOSAL IMMUNITY: A POTENTIAL BRIDGE TO AUTOIMMUNITY

The human mucosa includes its own highly evolved immune system composed of specialized epithelial cells and an array of resident inflammatory cells, including neutrophils, antigen-presenting cells, and lymphocytes, the latter interacting with epithelial cells to maintain normal mucosal homeostasis.¹⁶ In addition to harboring host inflammatory cells, mucosal tissues support a rich and diverse microbiome that is increasingly understood to play a role in host immunity and, in some cases, autoimmunity.¹⁷ Mucosal tissues provide the first order of protection for the host against a variety of environmental onslaughts that include exposures, such as cigarette smoke and invading pathogens, both of which have been implicated as RA risk factors.¹⁸⁻²¹ For instance, smoking demonstrates a dose-dependent relationship with the risk of autoantibody-positive RA, accounting for approximately 1 of every 6 new cases.¹⁸ This risk is highest among the genetically predisposed, in particular those carrying the *HLA-DRB1* shared epitope,^{22,23} the strongest genetic risk factor in patients with RA. Furthermore, smoking has been associated with

an increase in tissue accumulation of both citrullinated and carbamylated proteinms.^{24,25} In addition, IgM and IgG anti-MAA antibody isotypes are found in higher concentrations among current smokers, although only the increases in IgM levels reached statistical significance.⁷ Epithelial cells, which are at the forefront of mucosal protection, frequently harbor proteins bearing the same posttranslational modifications that are targeted by disease-specific autoantibodies.²⁶

Disease-related autoantibodies in patients with RA include the IgA isotypes of RF, ACPA, and anti-MAA,^{7,27} which is notable given the density of IgA-producing plasma cells found in the mucosal immune system²⁸ and ectopic lymphoid tissues that can develop at mucosal surfaces and act to present antigen and drive mature local and systemic immunologic responses.^{29,30} Janssen et al³¹ recently demonstrated associations of mucosal inflammation with low-level increases in RA-associated autoantibodies (including RF and ACPAs) in patients without articular signs or symptoms of RA but with the alternative diagnoses of chronic bronchiectasis, cystic fibrosis, or periodontitis (PD). The authors also observed increased serum concentrations of

IgA RF and IgA anti-cyclic citrullinated peptide (CCP) antibody (a commercial measure of ACPA) in patients with bronchiectasis. Positivity to IgA RF was increased among smokers regardless of underlying diagnosis. More recently, pulmonary bronchiectasis has been proposed as a model for the early induction of RA-related autoimmunity.³² Furthermore, Rangel-Moreno et al³³ demonstrated in patients with established articular RA and interstitial lung disease that ectopic lymphoid tissue in the lungs generated both RF and ACPAs. Taken together, these data suggest that the host protection offered by mucosal immunity might come at a cost, with mucosal inflammation acting as an initial nidus in the early events leading to RA.

MUCOSAL SITES IMPLICATED IN RA PATHOGENESIS

Lung

Pulmonary involvement is a well-recognized extra-articular complication in patients with RA, with manifestations related to interstitial lung disease, bronchiectasis, chronic obstructive pulmonary disease, pleurisy, and organizing pneumonia among others. The possibility that RA can be initiated in the lung is underscored by reports of pulmonary involvement preceding articular symptoms in seropositive patients. Fischer et al³⁴ recently examined 74 ACPA-positive patients presenting with respiratory symptoms in the absence of signs or symptoms of RA. Among the 33 subjects evaluated with high-titer ACPAs, 3 had articular symptoms of RA over a median follow-up of just over 1 year.

The relationship of airway inflammation with future disease risk has been explored extensively in subjects without evidence of RA but deemed to be at increased risk based on a positive family history or serum autoantibody positivity. Demoruelle et al³⁵ examined 42 subjects without evidence of inflammatory arthritis but with positive results for either ACPAs or 2 or more RF isotypes (autoantibody profiles associated with a substantially higher RA risk) along with 15 autoantibody-negative control subjects and 12 patients with early RA. All patients underwent high-resolution computed tomography (HRCT) lung imaging, the latter read in a blinded fashion by expert evaluators. Of note, 76% of the autoantibody-positive subjects had airway abnormalities, suggesting inflammatory disease detected on HRCT, compared with just 33% of autoantibody-negative control subjects ($P = .005$). In a separate study simultaneously collected serum samples and induced sputum were evaluated for RA-related autoantibodies in patients with early RA, healthy control subjects, and subjects at increased risk of future RA (serum seropositivity or positive family history of RA).³⁶ Autoantibodies examined included RF isotypes and ACPA, the latter including both the commercial second-generation anti-CCP antibody and the anti-CCP3.1 assay, which is more sensitive in detecting IgA ACPAs. At least 1 disease-related autoantibody was detected in sputum of 39% of the at-risk seronegative subjects, 65% of the seropositive at-risk subjects, and the vast majority (86%) of those with early RA. Intriguingly, among seronegative at-risk subjects, anti-CCP3.1 results were positive more often in sputum than in serum (6/23 in sputum vs 0/23 in serum, $P = .03$). Moreover, among subjects with detectable autoantibodies in sputum, the ratio of autoantibody to total immunoglobulin was higher in sputum than in sera, suggesting that these autoantibodies are either produced or sequestered locally in lung tissues during preclinical phases of RA.

Similar results have been obtained from patients with untreated ACPA-positive RA.³⁷ Using both HRCT and bronchoalveolar lavage (BAL) specimens, Reynisdottir et al³⁸ found that almost two thirds of ACPA-positive patients with RA had evidence of parenchymal lung abnormalities, as determined by using HRCT, compared with just 37% of ACPA-negative subjects ($P < .05$), a difference that was independent of smoking status. Moreover, ACPA concentrations were generally greater in BAL versus paired serum samples, and similar to the aforementioned at-risk subjects, the ratio of IgG ACPA to total IgG was higher in BAL fluid than serum. Examining large bronchial mucosal biopsy specimens in a small subset of patients with RA, this group further demonstrated increased lymphocyte infiltration and inducible bronchus-associated lymphoid tissues in approximately half of the ACPA-positive patients but only 1 of 6 ACPA-negative patients and only 1 of 9 healthy control subjects.³⁸ These observations again support the concept of local ACPA production in the airways or its local sequestration as an early event in RA etiopathogenesis.

Although the precise mechanisms underpinning the relationship of airway inflammation with autoantibody generation and heightened RA risk have not been fully elucidated, several investigations have provided insight. In their formative report Klareskog et al³⁹ identified citrullinated antigen in BAL fluids obtained from smokers, a finding that was absent among non-smokers. These observations were replicated in a larger study, also demonstrating increased PAD expression in the lungs.²⁴ These observations support the hypothesis that long-term exposure to cigarette smoke and possibly other environmental stimuli, such as chronic infection, can accelerate the formation of autoantigen and eventual ACPA production locally.²⁴

More recently, Quirke et al³² examined bronchiectasis as a model for how chronic pulmonary bacterial infection could serve as an impetus for early autoimmunity leading to RA. In this study investigators examined 122 patients with bronchiectasis alone and 50 patients with RA without lung disease, as well as both diseased ($n = 87$) and healthy ($n = 79$) control subjects. Patients with bronchiectasis were more often positive for RF and ACPA targeting citrullinated fibrinogen ($P < .05$). Of note, in contrast to patients with RA who demonstrate a citrulline-specific ACPA repertoire, patients with bronchiectasis in the absence of RA had increased autoantibody to native (unmodified) peptides that are bound by ACPA after citrullination, raising the question of whether initial autoimmune responses might be to native antigens, with later development of responses to citrullinated antigens being a key factor in transitioning from mucosal-limited autoimmunity to articular disease in patients with RA.

Associations of early airway inflammation with the generation of other disease-related autoantibodies, namely anti-CarP, anti-MAA, and anti-PAD4 antibodies, have been less well studied. Animal models with exposure to a combination of alcohol and cigarette smoke demonstrate increased airway expression of MAA adducts.⁴⁰ Whether these MAA adducts colocalize with citrullinated antigen (as demonstrated to occur in synovial tissues of patients with RA⁷) or whether these lead to local anti-MAA antibody production in the human lung has yet to be shown. Likewise, in patients with established RA, the presence of circulating anti-PAD4 antibody has been strongly associated with interstitial lung disease, particularly among cigarette smokers.⁴¹ Whether anti-PAD4 antibody is produced locally or acts as an etiologic link between early airway inflammation and the development of RA is unknown. In their examination of autoantibody expression and

mucosal inflammation, Janssen et al³¹ found no association between anti-CarP antibody with bronchiectasis in the absence of RA.

Overall, these studies support that RA-related autoimmunity can be generated in the lung in some subjects who do not have articular RA, perhaps as part of a natural response to local factors. Conversely, the observation of increased serum RA-related autoantibody levels in some at-risk subjects in the absence of increases in sputum levels suggests that sites other than the lung might be generating these autoantibodies.³⁶ In the subset of patients with established RA and synovitis, this alternative site could be the synovium, as supported by findings by several research groups of intra-articular generation of ACPAs in patients with established RA.^{42,43} However, in at-risk subjects without inflammatory arthritis, this could indicate other mucosal sites are generating autoimmunity.

Key to our understanding will be future investigations identifying the exact nature of the autoantigens produced by local airway inflammation. In a preliminary report Ytterberg et al⁴⁴ examined both synovial and lung tissues from patients with RA, showing that 2 identical autoantigens (2 citrullinated vimentin peptides) were detected in both tissues in a majority of patients. In addition to replicating these results in larger and more diverse populations, additional studies will be needed to characterize the biologic pathways involved in antigen processing and presentation, events involved in the transition from local mucosal production of autoimmunity to clinically detectable synovitis and definable articular RA, and potential interventions that might be leveraged with this improved understanding.

Oral cavity

Following the concept that other mucosal sites might be involved in the pathogenesis of RA, disorders characterized by chronic oral inflammation are also increased in patients with RA. In case-control studies chronic PD has consistently been shown to be more common in patients with RA than in comparator populations.^{19,20,45-52} In the largest such study to date, our group recently found that ACPA-positive patients with RA (n = 287) had a 50% higher prevalence of PD than patients with osteoarthritis (n = 330), even after accounting for factors such as oral hygiene, cigarette smoking, sex, race/ethnicity, medications, and self-reported diabetes mellitus, among other factors examined.¹⁹ As in at least 1 other study, this association was strongest among those with ACPA-positive disease.⁴⁷ Moreover, compared with those without PD, patients with RA and PD had higher serum concentrations of ACPAs and RF and had greater articular damage on hand and wrist radiographs.¹⁹

Similar to the pulmonary mucosa, evolving evidence suggests that the inflamed periodontium can serve as a reservoir of RA-related autoantigens, with at least 1 report demonstrating that citrullinated proteins are expressed in the periodontia of patients with PD in a pattern that is markedly similar to that observed in synovium from patients with RA.²⁶ Although it is hypothesized that other posttranslational modifications that drive RA-related autoimmunity are expressed in oral tissues,⁵³ investigations definitively demonstrating the presence of MAA-modified adducts or CarPs have yet to be systematically undertaken. In addition to the expression of autoantigen, autoantibodies also appear to be produced or sequestered locally in PD. RF has been demonstrated in the gingiva, subgingival plaque, and saliva of patients with PD,

even in the absence of RA.⁵⁴ In addition to detecting citrullinated antigen, Harvey et al⁵⁵ found an upregulation of PAD2 and PAD4 isoforms in PD-affected tissues and detected expression of ACPAs in the gingival crevicular fluid of some patients.

In addition to the direct contributions from innate immune responses,¹⁰ there has been substantial speculation regarding the role of select bacteria that cause PD in the generation of RA-related autoantigens. Chief among these is *Porphyromonas gingivalis*, the only prokaryote known to express a PAD-like enzyme as a virulence factor.⁵⁶ A prevailing hypothesis involves the bacteria-mediated cleavage of proteins by gingipains, exposing terminal arginine residues as a substrate for bacterial citrullination. Consistent with this model, *P. gingivalis* has the capacity to citrullinate both fibrinogen and, to a lesser extent, enolase,⁵⁷ peptide antigens that have been implicated in RA pathogenesis when citrullinated.^{58,59} Indeed, enolase expressed by *P. gingivalis* shows substantial homology with human enolase,⁵⁸ raising the possibility of molecular mimicry driving tolerance loss.

In addition to catalyzing citrullination, infection with *P. gingivalis* has been shown in animal models to exacerbate arthritis phenotypes and promote T_H17 responses, fostering a more ideal milieu for autoimmunity.^{60,61} Patients with RA have been shown to have higher serum concentrations of anti-*P. gingivalis* antibody compared with control subjects,^{62,63} although others have not replicated this finding.^{20,64} Among those with RA, circulating ACPAs show significant, although modest, correlations with levels of antibody to *P. gingivalis* but not with other oral pathogens.¹⁹ Patients positive for ACPAs, RF, or both who are at increased risk of RA also have higher concentrations of anti-*P. gingivalis* antibody.^{65,66} Because lysine and arginine residues targeted by bacterially expressed gingipains exist in the Fc region of IgG,⁶⁷ it has been suggested that modification of these domains leads to targeting and binding with RF.⁶⁸ Because other oral bacterial species also express gingipains, associations of PD with RF could be mediated by multiple pathogens. Indeed, RF seropositivity in patients with PD has been associated with increased antibody levels to *Fusobacterium nucleatum* and *Actinobacillus* (now *Aggregatibacter*) *actinomycetemcomitans*, which are known PD pathogens.⁶⁸

If PD or infection leading to PD is causally related to RA risk, then it follows that PD treatment could decrease this risk and mitigate autoimmune responses. Although the effect of PD treatment on RA outcomes has been examined in several small, single-center studies, few of these have examined the effect of interventions on the expression of autoantigens or autoimmunity.^{69,70} In at least 1 study patients with RA undergoing PD treatment experienced numeric decreases in circulating RF levels, although this change did not reach statistical significance.⁷¹ In another small study patients with RA undergoing PD intervention demonstrated significant decreases in RA disease activity along with decreases in levels of anti-*P. gingivalis* antibody and circulating citrullinated antigen.⁷² Whether such an intervention decreases or prevents the generation of pathogenic ACPAs remains unknown.

Other mucosal surfaces

Although less well studied, other sites of mucosal inflammation have also been examined in relation to RA risk. Scher et al,⁷³ for example, recently examined the composition of the gut microbiome in patients with RA and control subjects, observing a

relative expansion of *Prevotella copri* in patients with new-onset treatment-naïve disease. These findings come in the wake of several animal studies demonstrating the ability of intestinal microbes to induce arthritis. For example, the arthritis phenotype characteristic of the K/BxN mouse model is greatly abrogated with elimination of intestinal microbes, although colonization with a single microorganism (segmented filamentous bacteria) recapitulated the arthritis and induced potent T_H17 inflammatory responses.⁷⁴ Inflammation of the genitourinary tract, namely that related to the gram-negative bacteria *Proteus mirabilis*, has also been implicated in patients with RA.⁷⁵ In a preliminary report examining a large cohort of first-degree relatives of RA probands (none of whom had RA), women with a history of intrauterine device use were almost 3 times as likely as women without intrauterine device use to be ACPA positive, even after adjusting for age, race, smoking status, and *HLA-DRB1* shared epitope status.⁷⁶ Although preliminary, these results implicate endometrial inflammation as yet another potential mucosal site of involvement in the early evolution of RA.

CONCLUSIONS

Taken together, these studies support a model through which select environmental triggers, such as smoking or microbial pathogens, result in local mucosal inflammation targeting the lung, oral cavity, and perhaps other sites (Fig 1). In turn, this inflammation is associated with the generation of neoantigens and, among genetically susceptible subjects (eg, those carrying *HLA-DRB1* shared epitope or other genetic risk factors), the subsequent production of local RA-related autoantibodies. With the passage of time and local expression of synovial neoantigens, accompanied by epitope spreading and the requisite generation of higher-affinity autoantibody, a select proportion of subjects transition from an initial “spark” comprised of localized mucosal inflammation to the “fire” in patients with RA that is characterized by polyarticular synovitis, progressive joint destruction, and systemic inflammation.

Notably, a majority of findings thus far implicating mucosal inflammation as an initial spark in patients with RA have focused on the oral cavity and the lung. Although these studies have provided us with intriguing data and support that mucosal surfaces can generate RA-related autoimmunity, much more remains to be done. This includes more in-depth exploration of specific mechanisms through which RA-related autoimmunity could be generated at these mucosal surfaces. In addition, other mucosal surfaces need to be explored because it could well be that the mucosal sites that trigger RA-related autoimmunity vary across subjects. Specifically, given the female preponderance of RA and potential differences in environmental risk factors across sexes (eg, in some studies smoking appears to be a stronger risk factor for RA in men than women⁷⁷), we might need to focus on different mucosal sites based on sex or other factors. To do this, we need to develop informative cohorts that can be used to study the earliest phases of RA development. We also need to identify techniques to safely and accurately assess mucosal immune and autoimmune responses, as well as develop robust and highly relevant animal models that can help to elucidate mucosal involvement in the generation of RA. Furthermore, we need to explore how initial mucosal autoimmunity transitions to articular disease. It is imperative that these issues be explored because ultimately understanding how mucosal processes drive RA-related

autoimmunity could lead to novel and effective preventive interventions for RA.

REFERENCES

1. Birnbaum H, Pike C, Kaufman R, Marynchenko M, Kidolezi Y, Cifaldi M. Societal cost of rheumatoid arthritis patients in the US. *Curr Med Res Opin* 2010;26:77-90.
2. Franklin EC, Holman HR, Muller-Eberhard HJ, Kunkel HG. An unusual protein component of high molecular weight in the serum of certain patients with rheumatoid arthritis. *J Exp Med* 1957;105:425-38.
3. Demoruelle MK, Parish MC, Derber LA, Kolfschoten JR, Hughes-Austin JM, Weisman MH, et al. Performance of anti-cyclic citrullinated Peptide assays differs in subjects at increased risk of rheumatoid arthritis and subjects with established disease. *Arthritis Rheum* 2013;65:2243-52.
4. Harris ML, Darrah E, Lam GK, Bartlett SJ, Giles JT, Grant AV, et al. Association of autoimmunity to peptidyl arginine deiminase type 4 with genotype and disease severity in rheumatoid arthritis. *Arthritis Rheum* 2008;58:1958-67.
5. Schellekens GA, Visser H, de Jong BA, van den Hoogen FH, Hazes JM, Breedveld FC, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum* 2000;43:155-63.
6. Shi J, Knevel R, Suwannalai P, van der Linden MP, Janssen GM, van Veelen PA, et al. Autoantibodies recognizing carbamylated proteins are present in sera of patients with rheumatoid arthritis and predict joint damage. *Proc Natl Acad Sci U S A* 2011;108:17372-7.
7. Thiele GM, Duryee MJ, Anderson DR, Klassen LW, Mohring SM, Young KA, et al. Malondialdehyde-acetaldehyde adducts and anti-malondialdehyde-acetaldehyde antibodies in rheumatoid arthritis. *Arthritis Rheumatol* 2015;67:645-55.
8. Thiele GM, Tuma DJ, Willis MS, Miller JA, McDonald TL, Sorrell MF, et al. Soluble proteins modified with acetaldehyde and malondialdehyde are immunogenic in the absence of adjuvant. *Alcohol Clin Exp Res* 1998;22:1731-9.
9. Tuma DJ, Kearley ML, Thiele GM, Worrall S, Haver A, Klassen LW, et al. Elucidation of reaction scheme describing malondialdehyde-acetaldehyde-protein adduct formation. *Chem Res Toxicol* 2001;14:822-32.
10. Darrah E, Andrade F. Editorial: citrullination, and carbamylation, and malondialdehyde-acetaldehyde! Oh my! Entering the forest of autoantigen modifications in rheumatoid arthritis. *Arthritis Rheumatol* 2015;67:604-8.
11. Gan RW, Trouw LA, Shi J, Toes RE, Huizinga TW, Demoruelle MK, et al. Anti-carbamylated protein antibodies are present prior to rheumatoid arthritis and are associated with its future diagnosis. *J Rheumatol* 2015;42:572-9.
12. Nielsen MM, van Schaardenburg D, Reesink HW, van de Stadt RJ, van der Horst-Bruinsma IE, de Koning MH, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380-6.
13. Sokolove J, Bromberg R, Deane KD, Lahey LJ, Derber LA, Chandra PE, et al. Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. *PLoS One* 2012;7:e35296.
14. de Hair MJ, van de Sande MG, Ramwadhoebe TH, Hansson M, Landewe R, van der Leij C, et al. Features of the synovium of individuals at risk of developing rheumatoid arthritis: implications for understanding preclinical rheumatoid arthritis. *Arthritis Rheumatol* 2014;66:513-22.
15. van de Stadt LA, Bos WH, Meursingh Reynders M, Wieringa H, Turkstra F, van der Laken CJ, et al. The value of ultrasonography in predicting arthritis in autoantibody positive arthralgia patients: a prospective cohort study. *Arthritis Res Ther* 2010;12:R98.
16. Cheroute H, Lambolez F, Mucida D. The light and dark sides of intestinal intra-epithelial lymphocytes. *Nat Rev Immunol* 2011;11:445-56.
17. Longman RS, Littman DR. The functional impact of the intestinal microbiome on mucosal immunity and systemic autoimmunity. *Curr Opin Rheumatol* 2015;27:381-7.
18. Criswell LA, Merlino LA, Cerhan JR, Mikuls TR, Mudano AS, Burma M, et al. Cigarette smoking and the risk of rheumatoid arthritis among postmenopausal women: results from the Iowa Women's Health Study. *Am J Med* 2002;112:465-71.
19. Mikuls TR, Payne JB, Yu F, Thiele GM, Reynolds RJ, Cannon GW, et al. Periodontitis and Porphyromonas gingivalis in patients with rheumatoid arthritis. *Arthritis Rheumatol* 2014;66:1090-100.
20. Scher JU, Ubeda C, Equinda M, Khanin R, Buischi Y, Viale A, et al. Periodontal disease and the oral microbiota in new-onset rheumatoid arthritis. *Arthritis Rheum* 2012;64:3083-94.
21. Silman A, Newman J, MacGregor A. Cigarette smoking increases the risk of rheumatoid arthritis. *Arthritis Rheum* 1996;39:732-5.

22. Kallberg H, Ding B, Padyukov L, Bengtsson C, Ronnelid J, Klareskog L, et al. Smoking is a major preventable risk factor for rheumatoid arthritis: estimations of risks after various exposures to cigarette smoke. *Ann Rheum Dis* 2011;70:508-11.
23. Kallberg H, Padyukov L, Plenge R, Ronnelid J, Gregersen PK, van der Helm-van Mil AHM, et al. Gene-gene and gene-environment interactions involving HLA-DRB1, PTPN22, and smoking in two subsets of rheumatoid arthritis. *Am J Hum Genet* 2007;80:867-75.
24. Makrygiannakis D, Hermansson M, Ulfgren AK, Nicholas AP, Zendman AJ, Eklund A, et al. Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. *Ann Rheum Dis* 2008;67:1488-92.
25. Wang Z, Nicholls SJ, Rodriguez ER, Kumm O, Horkko S, Barnard J, et al. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. *Nat Med* 2007;13:1176-84.
26. Nesse W, Westra J, van der Wal JE, Abbas F, Nicholas AP, Vissink A, et al. The periodontium of periodontitis patients contains citrullinated proteins which may play a role in ACPA (anti-citrullinated protein antibody) formation. *J Clin Periodontol* 2012;39:599-607.
27. Falkenburg WJ, van Schaardenburg D, Ooijevaar-de Heer P, Wolbink G, Rispen T. IgG subclass specificity discriminates restricted IgM rheumatoid factor responses from more mature ACPA-associated or isotype-switched IgA responses. *Arthritis Rheumatol* 2015 [Epub ahead of print] doi: 10.1002/art.39299.
28. McGhee JR, Fujishashi K. Inside the mucosal immune system. *PLoS Biol* 2012;10:e1001397.
29. Carragher DM, Rangel-Moreno J, Randall TD. Ectopic lymphoid tissues and local immunity. *Semin Immunol* 2008;20:26-42.
30. Cesta MF. Normal structure, function, and histology of mucosa-associated lymphoid tissue. *Toxicol Pathol* 2006;34:599-608.
31. Janssen KM, de Smit MJ, Brouwer E, de Kok FA, Kraan J, Altenburg J, et al. Rheumatoid arthritis-associated autoantibodies in non-rheumatoid arthritis patients with mucosal inflammation: a case-control study. *Arthritis Res Ther* 2015;17:174.
32. Quirke AM, Perry E, Cartwright A, Kelly C, De Soyza A, Eggleton P, et al. Bronchiectasis is a model for chronic bacterial infection inducing autoimmunity in rheumatoid arthritis. *Arthritis Rheumatol* 2015;67:2335-42.
33. 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;116:3183-94.
34. Fischer A, Solomon JJ, du Bois RM, Deane KD, Olson AL, Fernandez-Perez ER, et al. Lung disease with anti-CCP antibodies but not rheumatoid arthritis or connective tissue disease. *Respir Med* 2012;106:1040-7.
35. Demoruelle MK, Weisman MH, Simonian PL, Lynch DA, Sachs PB, Pedraza IF, 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;64:1756-61.
36. Willis VC, Demoruelle MK, Derber LA, Chartier-Logan CJ, Parish MC, Pedraza IF, et al. Sputum autoantibodies in patients with established rheumatoid arthritis and subjects at risk of future clinically apparent disease. *Arthritis Rheum* 2013;65:2545-54.
37. Reynisdottir G, Karimi R, Joshua V, Olsen H, Hensvold AH, Harju A, et al. Structural changes and antibody enrichment in the lungs are early features of anti-citrullinated protein antibody-positive rheumatoid arthritis. *Arthritis Rheumatol* 2014;66:31-9.
38. Reynisdottir G, Joshua V, Hensvold AH, Klareskog L, Catrina AI. Characterization of lung inflammation in the lungs of early rheumatoid arthritis [abstract]. *Arthritis Rheumatol* 2014;66(suppl):s434.
39. Klareskog L, Stolt P, Lundberg K, Kallberg H, Bengtsson C, Grunewald J, 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.
40. McCaskill ML, Kharbanda KK, Tuma DJ, Reynolds JD, DeVasure JM, Sisson JH, et al. Hybrid malondialdehyde and acetaldehyde protein adducts form in the lungs of mice exposed to alcohol and cigarette smoke. *Alcohol Clin Exp Res* 2011;35:1106-13.
41. Giles JT, Darrah E, Danoff S, Johnson C, Andrade F, Rosen A, et al. Association of cross-reactive antibodies targeting peptidyl-arginine deiminase 3 and 4 with rheumatoid arthritis-associated interstitial lung disease. *PLoS One* 2014;9:e98794.
42. Snir O, Widhe M, Hermansson M, von Spee C, Lindberg J, Hensen S, et al. Antibodies to several citrullinated antigens are enriched in the joints of rheumatoid arthritis patients. *Arthritis Rheum* 2010;62:44-52.
43. Masson-Bessiere C, Sebbag M, Durieux JJ, Nogueira L, Vincent C, Girbal-Neuhauser E, et al. In the rheumatoid pannus, anti-filaggrin autoantibodies are produced by local plasma cells and constitute a higher proportion of IgG than in synovial fluid and serum. *Clin Exp Immunol* 2000;119:544-52.
44. Ytterberg AJ, Joshua V, Reynisdottir G, Tarasova NK, Rutishauser D, Ossipova E, et al. Shared immunological targets in the lungs and joints of patients with rheumatoid arthritis: identification and validation. *Ann Rheum Dis* 2015;74:1772-7.
45. de Pablo P, Dietrich T, McAlindon TE. Association of periodontal disease and tooth loss with rheumatoid arthritis in the US population. *J Rheumatol* 2008;35:70-6.
46. de Smit MD, Westra J, Vissink A, Doornbos-van der Meer B, Brouwer E, van Winkelhoff AJ. Periodontitis in established rheumatoid arthritis patients: a cross-sectional clinical, microbiological and serological study. *Arthritis Res Ther* 2012;14:R222.
47. Dissick A, Redman RS, Jones M, Rangan BV, Reimold A, Griffiths GR, et al. Association of periodontitis with rheumatoid arthritis—a pilot study. *J Periodontol* 2010;81:223-30.
48. Kasser UR, Gleissner C, Dehne F, Michel A, Willershausen-Zonnchen B, Bolten WW. Risk for periodontal disease in patients with longstanding rheumatoid arthritis. *Arthritis Rheum* 1997;40:2248-51.
49. Malmstrom M, Caloni PE. Teeth loss and the inflammation of teeth-supporting tissues in rheumatoid disease. *Scand J Rheumatol* 1975;4:49-55.
50. Mercado FB, Marshall RI, Klestov AC, Bartold PM. Relationship between rheumatoid arthritis and periodontitis. *J Periodontol* 2001;72:779-87.
51. Pischon N, Pischon T, Kroger J, Gulmez E, Kleber BM, Bernimoulin JP, et al. Association among rheumatoid arthritis, oral hygiene, and periodontitis. *J Periodontol* 2008;79:979-86.
52. Tolo K, Jorkjend L. Serum antibodies and loss of periodontal bone in patients with rheumatoid arthritis. *J Clin Periodontol* 1990;17:288-91.
53. Bright R, Proudman SM, Rosenstein ED, Bartold PM. Is there a link between carbamylation and citrullination in periodontal disease and rheumatoid arthritis? *Med Hypotheses* 2015;84:570-6.
54. Gargiulo AV, Robinson J, Toto PD, Gargiulo AW. Identification of rheumatoid factor in periodontal disease. *J Periodontol* 1982;53:568-77.
55. 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 Periodontol Res* 2013;48:252-61.
56. Rosenstein ED, Greenwald RA, Kushner LJ, Weissmann G. Hypothesis: the humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis. *Inflammation* 2004;28:311-8.
57. Wegner N, Wait R, Sroka A, Eick S, Nguyen KA, Lundberg K, et al. Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and alpha-enolase: implications for autoimmunity in rheumatoid arthritis. *Arthritis Rheum* 2010;62:2662-72.
58. Lundberg K, Kinloch A, Fisher BA, Wegner N, Wait R, Charles P, et al. Antibodies to citrullinated alpha-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. *Arthritis Rheum* 2008;58:3009-19.
59. Snir O, Widhe M, von Spee C, Lindberg J, Padyukov L, Lundberg K, et al. Multiple antibody reactivities to citrullinated antigens in sera from rheumatoid arthritis patients—association with HLA-DRB1 alleles. *Ann Rheum Dis* 2009;68:736-43.
60. Cantley MD, Haynes DR, Marino V, Bartold PM. Pre-existing periodontitis exacerbates experimental arthritis in a mouse model. *J Clin Periodontol* 2011;38:532-41.
61. Marchesan JT, Gerow EA, Schaff R, Taut AD, Shin SY, Sugai J, et al. *Porphyromonas gingivalis* oral infection exacerbates the development and severity of collagen-induced arthritis. *Arthritis Res Ther* 2013;15:R186.
62. Mikuls TR, Payne JB, Reinhardt RA, Thiele GM, Maziarz E, Cannella AC, et al. Antibody responses to *Porphyromonas gingivalis* (*P. gingivalis*) in subjects with rheumatoid arthritis and periodontitis. *Int Immunopharmacol* 2009;9:38-42.
63. Oğrendik M, Kokino S, Ozdemir F, Bird PS, Hamlet S. Serum antibodies to oral anaerobic bacteria in patients with rheumatoid arthritis. *MedGenMed* 2005;7:2.
64. Seror R, Le Gall-David S, Bonnaure-Mallet M, Schaefferbeke T, Cantagrel A, Minet J, et al. Association of anti-*Porphyromonas gingivalis* antibody titers with nonsmoking status in early rheumatoid arthritis: results from the prospective french cohort of patients with early rheumatoid arthritis. *Arthritis Rheumatol* 2015;67:1729-37.
65. Hitchon CA, Chandad F, Ferucci ED, Willemze A, Ioan-Facsinay A, Van der Woude D, et al. Antibodies to *Porphyromonas gingivalis* are associated with anti-citrullinated protein antibodies in RA patients and their relatives. *J Rheumatol* 2010;37:1105-12.
66. Mikuls TR, Thiele GM, Deane KD, Payne JB, O'Dell JR, Yu F, et al. *Porphyromonas gingivalis* and disease-related autoantibodies in Individuals at increased risk for future rheumatoid arthritis. *Arthritis Rheum* 2012;64:3522-30.

67. Bonagura VR, Artandi SE, Davidson A, Randen I, Agostino N, Thompson K, et al. Mapping studies reveal unique epitopes on IgG recognized by rheumatoid arthritis-derived monoclonal rheumatoid factors. *J Immunol* 1993;151:3840-52.
68. The J, Ebersole JL. Rheumatoid factor from periodontitis patients cross-reacts with epitopes on oral bacteria. *Oral Dis* 1996;2:253-62.
69. Kaur S, Bright R, Proudman SM, Bartold PM. Does periodontal treatment influence clinical and biochemical measures for rheumatoid arthritis? A systematic review and meta-analysis. *Semin Arthritis Rheum* 2014;44:113-22.
70. Payne JB, Golub LM, Thiele GM, Mikuls TR. The link between periodontitis and rheumatoid arthritis: a periodontist's perspective. *Curr Oral Health Rep* 2015;2:20-9.
71. Ribeiro J, Leao A, Novaes AB. Periodontal infection as a possible severity factor for rheumatoid arthritis. *J Clin Periodontol* 2005;32:412-6.
72. Okada M, Kobayashi T, Ito S, Yokoyama T, Abe A, Murasawa A, et al. Periodontal treatment decreases levels of antibodies to *Porphyromonas gingivalis* and citrulline in patients with rheumatoid arthritis and periodontitis. *J Periodontol* 2013;84:e74-84.
73. Scher JU, Szczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, et al. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. *Elife* 2013;2:e01202.
74. Wu HJ, Ivanov II, Darce J, Hattori K, Shima T, Umesaki Y, et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. *Immunity* 2010;32:815-27.
75. Ebringer A, Rashid T. Rheumatoid arthritis is caused by Proteus: the molecular mimicry theory and Karl Popper. *Front Biosci (Elite Ed)* 2009;1:577-86.
76. Khatter S, Parish M, Feser ML, Kolfenbach JR, Gan RW, Weisman M, et al. Contraceptive factors are associated with serum antibodies to citrullinated protein antigens in women at elevated risk for future rheumatoid arthritis [abstract] . *Arthritis Rheumatol* 2014;66(suppl):s2929.
77. Krishnan E, Sokka T, Hannonen P. Smoking-gender interaction and risk for rheumatoid arthritis. *Arthritis Res Ther* 2003;5:R158-62.