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When and Where Does Inflammation Begin in Rheumatoid Arthritis?

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Abstract

Purpose of review—The etiology of rheumatoid arthritis (RA), as well as the timing and anatomic site at which RA-related autoimmunity is initiated, is currently unknown. An improved understanding of the initial steps in the development of RA would provide insights into disease pathogenesis that could ultimately lead to more effective treatments and/or novel preventive strategies in RA.

Recent findings—Systemic inflammation and autoimmunity in RA begin long before the onset of detectable joint inflammation. Emerging data suggest that RA-related autoimmunity may be initiated at a mucosal site years before the onset of joint symptoms. The candidate sites of origin include the oral, lung and gastrointestinal mucosa, as data consistent with this hypothesis have been generated for each location. Individual patients may undergo initiation events at unique sites, but still converge on similar joint findings as the disease process evolves.

Summary—Further investigations are needed to determine when and where RA begins, including comprehensive prospective studies of individuals in the preclinical period of RA that can provide insight into the relationship between mucosal inflammation, RA-related autoantibody generation and subsequent joint inflammation in RA.

Keywords

rheumatoid arthritis; preclinical; autoimmunity; inflammation; etiology

INTRODUCTION

Treatment strategies in rheumatoid arthritis (RA) have rapidly advanced over the past decade to include earlier identification and initiation of treatment in clinically classifiable RA. While this approach has led to improved outcomes of joint damage and disability, the number of RA patients that achieve sustained drug-free remission remains low[1-3]. Therefore, in order to “cure” RA and prevent joint damage, interventions prior to the onset of joint inflammation are very likely to be necessary. Unfortunately, a major limitation to

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developing effective preventive strategies for RA has been a lack of ability to detect and classify individuals that are at high risk for RA, as well as limitations in our knowledge of the mechanisms of disease development, including identification of when and at what anatomic site RA begins. However, in recent years, there has been a greatly increased understanding of the mechanisms of early RA development. Herein we will review these data, with a focus on when and where RA may begin, as well as identify areas of additional study that are required in this area.

PRECLINICAL RA

It is well-established that RA-related autoantibodies can be elevated in the serum on-average 3-5 years (and in some cases >10 years) prior to the onset of joint inflammation in RA. This finding is supported by data from multiple prospective and retrospective studies evaluating samples that were collected and stored prior to the onset of clinically apparent RA. These RA-related autoantibodies are listed in Table 1 and are highly specific for future disease [4-12, 15]. This period of autoimmunity in the absence of classifiable RA has been termed 'preclinical RA'.

Studies of autoantibodies in the preclinical period of RA have provided insights into the evolution of autoimmunity in RA. For example, autoantibody levels rise during the preclinical period as time to RA diagnosis decreases[6,7]. Additionally, there are changes in autoantibody characteristics during preclinical RA, including isotype, avidity, glycosylation and epitope spreading. Nielsen and colleagues recently demonstrated that a doubling of serum rheumatoid factor (RF)-IgM level was associated with a >3 fold increased risk of developing RA[11], and Ercan and colleagues demonstrated that as the time to diagnosis approaches in preclinical RA, there is increasing aberrant glycosylation that promotes complement activation and Fc receptor engagement [16]. Suwannalai and colleges demonstrated that the avidity of antibodies to citrullinated protein antigens (ACPAs) increases over time in preclinical RA, although this process appears to halt once clinically apparent disease is present, a finding that suggests there is a threshold that must be reached prior to onset of synovitis [17]. Furthermore, using a bead-based array to test antibodies to multiple individual citrullinated proteins/peptides, Sokolove and colleagues demonstrated that there is epitope spreading in the preclinical RA period [13]. Another study by Brink and colleagues evaluated individual ACPAs in preclinical RA samples, and they demonstrated that an increased number of positive ACPAs had a higher specificity for future RA[14]. Similar to what was seen in the Sokolove study, Brink and colleagues also found that systemic autoimmunity in RA was initially restricted to a small number of ACPAs that expanded as time to RA diagnosis approached. Of interest, the ACPAs detected earliest in the preclinical period were found to be the least stable in longitudinal samples. Other studies have similarly demonstrated that autoantibody levels and reactivity may fluctuate over time, including during the preclinical RA period[18,19].

In addition to autoantibodies, multiple studies have identified abnormalities of biomarkers that are reflective of systemic inflammation during the preclinical RA period, including circulating cytokines, chemokines, and variably C-reactive protein [13, 20-23]. A recent study by Hughes-Austin and colleagues found an elevated cytokine score that encompasses

multiple inflammatory cytokines was associated with elevations of serum RA-related autoantibodies in unaffected first-degree relatives (FDRs) of probands with RA[20]. Furthermore, these inflammatory biomarker dynamics may predict the likelihood and timing of future RA. For example, Deane and colleagues found in a study using preclinical US Military samples that an increasing number of elevated cytokines/chemokines was associated with a shorter duration to RA diagnosis[22]. Using the same sample set, Sokolove et al found that an increased number of ACPAs along with elevated cytokines was 58% sensitive and 87% specific for a diagnosis of RA within 2 years [13].

However, the temporal relationship between elevations of autoantibodies and abnormalities of systemic inflammation during the preclinical RA period has been difficult to clarify. The Sokolove study found that increasing number of ACPAs preceded the timing of increasing levels of systemic inflammatory cytokines [13]. However, in a cross-sectional study, El-Gabalawy and colleagues found cytokine/chemokine elevations in Native American FDRs in absence of autoantibodies[24].

Overall these findings suggest that there is a period of preclinical RA development during which there is an initial restricted immune response that expands over time in regard to magnitude and breadth of responses. In particular for ACPA-related autoimmunity, targets of autoimmunity may change over time, with some early antigens perhaps reflecting initial autoimmunity, and later antigens representing epitope spreading, and potentially reactivity to more pathogenic targets. Although it is not yet clear if certain inflammatory pathways precede the development of autoimmunity, it is reasonable that there is initially some degree of local or systemic inflammation that is associated with the early development of autoimmunity in RA, and then both inflammatory and autoimmune processes expand over time until clinically apparent arthritis develops.

Where does RA begin?

Based on the data discussed above, systemic inflammation and autoimmunity precede the onset of clinically classifiable RA; however, it is not clear where these processes initially develop in RA. While plasma cells in the synovium have been shown to generate autoantibodies in patients with established RA[25,26], studies evaluating the joint during the preclinical period have generally found that the synovium does not appear to be affected in this early stage of RA development. Specifically, van de Sande and colleagues found no histologic or magnetic resonance imaging (MRI) evidence of synovial inflammation in the knee in subjects with serum RA-related autoantibodies without clinically-evident synovitis[27]. In contrast, subclinical joint inflammation was reported using ultrasound, MRI and positron emission tomography (PET) imaging in a small number of subjects with serum ACPAs, joint symptoms of ‘arthralgias’, and no clinically-evident synovitis[28-30]. However, the specificity of these imaging findings is uncertain, and the majority of the ACPA positive subjects that were evaluated in these studies had no clinical or imaging evidence of synovitis, including several that later developed synovitis classifiable as RA. Overall, these data suggest that the initial inflammation and autoimmunity in RA begins outside of the joints.

If RA-related autoimmunity develops outside of the joints, where is that site? Several lines of evidence support that RA-related autoimmunity may originate at a mucosal site (See Table 2), and an overview of this hypothesis is depicted in Figure 1. In particular, IgA is the predominant antibody of the mucosal immune system, and data demonstrate IgA-ACPAs are elevated and highly specific for RA in preclinical and early RA subjects [38-40]. Furthermore, Barra and colleagues found that 41% of serum ACPA positivity in unaffected FDRs of probands with RA was IgA[40]. Additionally, as discussed below, findings related to specific mucosal sites including the oral cavity, the lungs and the gut suggest that environmental factors that may preferentially affect the mucosa appear to play an important role in the early pathogenesis of RA.

It is also of note that the etiology of other rheumatic diseases support the generation of autoimmunity associated with mucosal processes. For example, the etiology of rheumatic fever is well-established to result from a dysregulated immune response to *Streptococcus* infection of the pharyngeal mucosa that results in autoimmune-mediated injury of other tissues, including the joints [41]. In addition, reactive arthritis is another systemic inflammatory arthritis that can be initiated by infection and inflammation at a mucosal site (i.e. the gastrointestinal or genitourinary mucosa)[42].

Oral mucosa and RA-related autoimmunity—In recent years, the oral mucosa, specifically the gingiva and periodontal regions, has been studied as a potential site for the origins of RA. In classifiable RA, there is an increased prevalence and severity of periodontitis that has been associated with systemic RA-related autoantibodies [43-46], and in subjects without classified RA, severe periodontitis has also been associated with RA-related autoantibodies [47]. In addition, *Porphyromonas gingivalis (P. ging)*, a microbe commonly involved in periodontitis, is uniquely found to express a peptidylarginine deiminase (PAD) enzyme capable of citrullinating human peptides/proteins [48,49]. Furthermore, in subjects without classified RA, Mikuls and colleagues identified an association between elevations of antibodies to *P. ging* and serum RA-related autoantibodies [31], and inflamed gingival tissue has been shown to express increased levels of PAD and citrullinated proteins [50,32]. Of interest, Harvey and colleagues also identified the presence of local anti-CCP antibodies in gingival crevicular fluid associated with gingivitis. However, despite these intriguing associations, a recent study by Scher and colleagues found that *P. ging* was associated with severity of periodontitis but not specifically associated with new-onset RA[51]. Rather, they found that *Prevotella* and *Leptotrichia* were expanded in new-onset RA, and *Anaeroglobus geminatus* was associated with RA-related autoantibody positivity.

As such, going forward, longitudinal studies are needed that can simultaneously evaluate the relationship between oral pathogens, local gingival autoantibody generation, systemic RA-related autoimmunity, and joint inflammation in order to better understand the role of the oral mucosa in the etiology of RA.

The lung and RA-related autoimmunity—Another mucosal surface that is a potential originating site of autoimmunity in RA is the lung. This possibility is supported by established data that demonstrate increased RA risk is associated with inhaled exposures

such as cigarette smoke [52-54], and a high prevalence of lung disease including airways inflammation has been identified in established RA[53,33]. Furthermore, Demoruelle and colleagues recently identified a higher prevalence of inflammatory airways disease by computed tomographic imaging in arthritis-free subjects (by joint examination and in a subset of subjects, by MRI) with serum RA-related autoantibodies compared to autoantibody negative matched controls [33]. Importantly, this finding was independent of prior or current cigarette smoking. Additionally, Fischer and colleagues found 80% of anti-CCP positive subjects with chronic lung disease and no joint symptoms had imaging evidence of airways inflammation [55]; furthermore, in a subset of these subjects that had a lung biopsy, 96% demonstrated histologic evidence of lung inflammation. Importantly, in these 2 studies, 5 subjects developed synovitis classifiable as RA during longitudinal follow-up, and all 5 had evidence of lung inflammation preceding the development of clinically apparent arthritis.

While these findings demonstrate that lung disease may precede articular disease in RA, it is not clear if that is because the lung is a site of initiation of RA-related autoimmunity, or an earlier target of the same autoimmune-mediated injury that affects the joints (the same argument can be made about oral inflammation and RA). However, recent findings from Willis and colleagues suggest that at least in some subjects, RA-related autoimmunity may originate in the lung [34]. In this study, a subset of arthritis-free subjects had RF and/or anti-CCP antibodies present in their sputum that were not present in their serum, or that were present in higher levels in their sputum compared to their serum, suggesting that in this subset, these RA-related autoantibodies are generated in the lung.

As for potential mechanisms of mucosal generation of autoimmunity, in patients with chronic RA-related lung disease that underwent biopsy, an increased prevalence of inducible bronchus associated lymphoid tissue (iBALT) was demonstrated in RA lung tissue compared to patients with other forms of lung disease [35]. Of interest, these areas of iBALT in RA patients included plasma cells generating RA-related autoantibodies. Thus, iBALT may represent a mechanism by which RA-related autoimmunity is generated in the lung. Similar areas of mucosal associated lymphoid tissue (MALT) may serve as a site for development of autoimmunity in other mucosal regions.

The gut and RA-related autoimmunity—To date, much of the data investigating the gastrointestinal mucosa in RA has focused on the gut microbiome. The gut microbiome is known to influence development of the innate and adaptive immune system, and may therefore also play a role in the development of autoimmunity [56,57]. In murine studies, specific alterations of gut bacteria can enhance or attenuate susceptibility to experimentally-induced arthritis [58-60. In humans, studies have identified differences in the gut microbiota, specifically differences in relative abundance of various microbes, in patients with classifiable RA compared to controls [36,37]. However, these studies have been unable to distinguish whether differences in gut microbial communities are a cause of inflammation in RA, the result of an underlying inflammatory environment that selects for survival of certain microbes, or whether the therapies used in RA are responsible for altering the gut microbial composition. Additional study of subjects prior to onset of joint inflammation will be

informative to understand the relationship between the gastrointestinal microbiome and the development RA.

In summary, there is compelling evidence linking the mucosa of the oral cavity, lung and gut with the initiation of RA. However, other sites such as the nasopharyngeal, genitourinary (GU) and cervicovaginal (CV) mucosa have similar biologic features, including the formation of MALT. They are also exposed to a variety of environmental factors that could trigger inflammation, and as such, may serve as potential sites for genetic-environmental immune reactions that could lead to RA. While controversial, associations between serologic evidence of infection with organisms such as *Proteus* species that can cause urinary tract infections suggest a potential role for GU inflammation and RA development[61,62]. In addition, CV inflammation could help to explain the strong association between female sex and RA. Also, it may be that different mucosal sites are responsible for generation of autoimmunity in different individuals that develop RA, and perhaps a key linkage between mucosal inflammation and RA is the development of systemic pathogenic autoimmunity to citrullinated antigens. These issues will require further well-designed human studies as well as relevant animal models of disease.

Immunologic mechanisms that may be involved in the mucosal initiation of RA

Along with considering when and where RA begins, it is also important to consider what immune processes are involved in the loss tolerance to citrullinated and other autoantigens, as well as how autoimmunity that may initially develop at an extra-articular site can move to the joint perhaps several years later, and result in synovitis. In particular, there are several potential immunologic mechanisms by which breaks in tolerance to self antigens occur at mucosal surfaces. For example, toll-like receptor (TLR) signaling is an innate immune response to micro-organisms and other factors that has also been implicated in autoimmune responses[63]. Specifically, TLR binding of endogenous nuclear material aberrantly released during apoptosis has been associated with development of autoimmunity in systemic lupus erythematosus[64], and similar mechanisms may be involved in RA as apoptosis is known to be associated with generation of citrullinated endogenous proteins through the actions of PADs[65]. Furthermore, microbial factors present at mucosal surfaces may lead to molecular mimicry, or inflammation that in turn generates autoantibodies. For example, certain organisms such as *P. ging* may citrullinate human tissues [48], or neutrophil migration to a mucosal site may lead to tissue citrullination due to release of PADs[65]. In addition, neutrophil extracellular traps (NETs) containing citrullinated peptides that are released by activated neutrophils may externalize these citrullinated peptides, thereby resulting in generation of ACPAs[66]. Other mechanisms may include an altered balance between regulatory and autoreactive T cells. In addition, elevations of RF, even in absence of articular RA, have been associated with mucosal inflammation such as in periodontal disease and non-RA lung disease (i.e. infection-related lung diseases such as cystic fibrosis and tuberculosis)[67-69]. In aggregate, these data suggest there may be a link between mucosal inflammation and the generation of RF as well as autoimmunity to citrullinated proteins/peptides.

Finally, there are additional immunologic mechanisms to consider that may be involved in the transition of autoimmunity from an initial extra-articular site to later involve the joints. Such possibilities include immune complex formation/deposition, shared antigenic targets between a site of initiation of RA-related autoimmunity and the joints, epitope spreading that leads to generation of autoimmunity directed to joint proteins, and migration of activated T cells. Specifically, Zhao and colleagues demonstrated circulating immune complexes containing citrullinated fibrinogen in subjects with classified RA as well as co-localization of fibrinogen, immunoglobulin and complement in RA joint tissue suggesting these circulating immune complexes can deposit in the synovium, resulting in complement-mediated synovitis[70]. In addition, Ytterberg and colleagues have identified a shared citrullinated vimentin protein in the lung and joint tissue of subjects with classifiable RA[71], and vaccination studies demonstrate that antigen-specific T cell responses initiated at one mucosal surface can traffic to other organs[72]. These mechanisms will be important to study in order to gain a clear understanding of how autoimmunity may move from one site to another in RA, especially because blocking this transition at the right time in RA development may lead to disease prevention.

Conclusion and future directions

The data and ideas reviewed herein illustrate recent advances in the understanding of when and where RA begins; however, further study is needed to gain a deeper understanding of the mechanisms of RA development, especially in relationship to the site of initiation of RA. Of importance, while some studies of preclinical RA have been prospective, many of the studies providing information regarding immunologic and inflammatory processes in the preclinical period have been retrospective biobank/serum repository studies. As such, they have certain limitations including temporal irregularity of collection of samples and lack of detailed clinical data from the time of sampling (i.e. did the subject really have synovitis at the time of sample collection?). With these limitations, it is difficult to determine the exact timing of onset and evolution of RA-related autoimmunity. Furthermore, such studies cannot evaluate subjects in real-time for biologic processes, such as mucosal inflammation, that may influence autoimmunity.

Therefore, a crucial part of advancing this knowledge will be prospective natural history studies that can evaluate subjects at-risk for RA in real time and allow for mechanistic studies of disease development. Several such natural history studies are underway and will likely provide valuable contributions to the understanding of preclinical RA. These include studies such as the Studies of the Etiology of RA (SERA) project, the North American Natives (NAN) study, and several research groups that have begun to evaluate FDRs in Korea, Sweden and Canada [40],73-76. In addition, preclinical RA research must advance to investigate specific immunologic mechanisms by which RA is generated at mucosal surface, or other potential sites, and results in joint disease. While some of these studies can be performed using biologic samples obtained from human subjects, it may be difficult to obtain adequate materials in a safe manner. Consequently, informative animal models will also be crucial to understanding specific pathways of disease development. However, driving these studies should be the hope that improved understanding of the specific mechanisms of RA development may lead to prevention strategies for this disease.

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Key points

- Systemic inflammation and autoimmunity are detectable years prior to the onset of detectable joint inflammation suggesting RA is initially generated outside of the joints.
- Several mucosal surfaces have the potential to be a site for initial immune dysregulation and autoantibody generation in RA.
- Additional investigations into the mechanisms by which inflammation and autoimmunity develop at a mucosal site are needed in order to understand the etiology of RA.
- An improved understanding of the earliest steps in the development of RA can lead to organ-targeted mechanism-specific strategies for RA prevention.

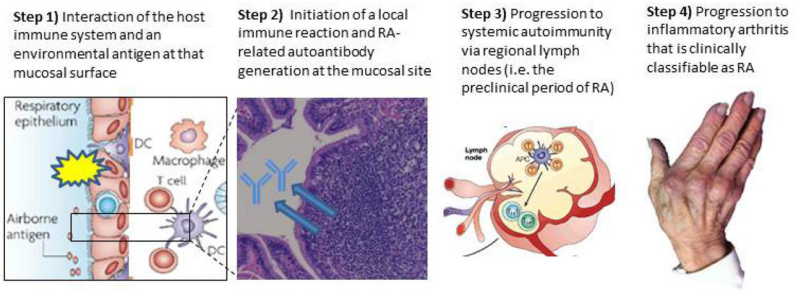

Figure 1. Hypothesis for the mucosal initiation of autoimmunity in RA

These pictures represent a step-by-step overview of the hypothesis that an environmental antigen interacting at a mucosal surface can result in a local immune-mediated inflammatory reaction. In some cases, this triggers an autoimmune response that is initially localized, but over time becomes systemic with circulating RA-related autoantibodies detected in the blood. Finally, after a period of circulating preclinical RA-related autoimmunity, joint inflammation clinically classifiable as RA may develop.

Table 1

Autoantibodies that are elevated during the preclinical period of rheumatoid arthritis

Autoantibody	Description
Rheumatoid factor (RF)[4-8,9,10,11]	• Autoantibody directed against the Fc portion of another immunoglobulin
Antibodies to citrullinated peptides (ACPA)[5-8, 12, 9, 10,13,14]	• Citrullination = post-translational amino acid modification of arginine to citrulline • Clinically characterized by antibodies to cyclic citrullinated peptides (CCP), an assay that includes several citrullinated protein/peptide targets
Antibodies to peptidyl arginine deiminase (PAD)[9]	• In humans, PAD2 and PAD4 are the enzymes responsible for the post-translational modification of arginine to citrulline. • Anti-PAD4 antibodies have been demonstrated in preclinical RA
Antibodies to carbamylated proteins (CarP)[10]	• Carbamylation = post-translational amino acid modification of lysine to homocitrulline • Unique process from citrullination, although homocitrulline and citrulline are structurally similar
Antibodies to mutated citrullinated vimentin (MCV)[15]	• The antigenic target is a modified vimentin that is both citrullinated and mutated

Table 2

Summary of studies supporting that RA originates at a mucosal site

Citation	Key points
Barra et al, 2013[19]	Case-control study that identified a high prevalence of serum ACPAs in unaffected first degree relatives (FDRs) of probands with RA (48%), and 41% of these ACPA positive FDRs were positive for IgA-ACPA
Van de Sande et al, 2011[27]	In this study, 13 subjects with serum anti-CCP and/or RF-IgM, joint symptoms of 'arthralgia', and no clinical synovitis had no evidence of synovial inflammation on knee MRI or joint biopsy
Mikuls et al, 2012 [31]	Cross-sectional study demonstrating antibodies to *Porphyromonas gingivalis* are associated with serum RF and/or anti-CCP in arthritis-free subjects at risk for future RA based on genetics and/or family history
Harvey et al, 2013 [32]	In a study of subjects without a clinical diagnosis of RA, periodontal inflammation was associated with increased citrullinated proteins, PAD2 and PAD4 as well as local anti-CCP antibodies in gingival crevicular fluid
Demoruelle et al, 2012 [33]	In a case-control study, arthritis-free subjects with serum RF and/or CCP positivity had a higher prevalence of airways disease on imaging compared to RF and CCP negative controls (76% vs. 33%, $p<0.01$)
Willis et al, 2013 [34]	Cross-sectional study of induced sputum demonstrating RF and ACPA positivity in the lung in classified RA. In several arthritis-free subjects, RF and ACPA levels in sputum suggested lung generation of these autoantibodies
Rangel-Moreno et al, 2006 [35]	In this histologic study of subjects with classified RA and related lung disease, inducible bronchus associated lymphoid tissue was more prevalent in RA and contained plasma cells generating RF and ACPA
Liu et al, 2013 [36]	In a case-control study, DNA sequencing identified an increased abundance of fecal Lactobacillus in early classified RA compared to healthy controls
Vaahtovuo et al, 2008 [37]	DMARD-naïve subjects with early classified RA had a decrease in the abundance of multiple fecal microbiota compared to fibromyalgia controls