RA: from risk factors and pathogenesis to prevention

Gene, environment, microbiome and mucosal immune tolerance in rheumatoid arthritis

Anca I. Catrina1, Kevin D. Deane2 and Jose U. Scher3

Abstract

RA is a complex multifactorial chronic disease that transitions through several stages. Multiple studies now support that there is a prolonged phase in early RA development during which there is serum elevation of RA-related autoantibodies including RF and ACPAs in the absence of clinically evident synovitis. This suggests that RA pathogenesis might originate in an extra-articular location, which we hypothesize is a mucosal site. In discussing this hypothesis, we will present herein the current understanding of mucosal immunology, including a discussion about the generation of autoimmune responses at these surfaces. We will also examine how other factors such as genes, microbes and other environmental toxins (including tobacco smoke) could influence the triggering of autoimmunity at mucosal sites and eventually systemic organ disease. We will also propose a research agenda to improve our understanding of the role of mucosal inflammation in the development of RA.

Key words: rheumatoid arthritis, environmental risk factors, genetic susceptibility, microbiome, break in tolerance.

Introduction

RA is one of the most common forms of inflammatory arthritis. Notable advances in the understanding of its pathogenesis have been achieved in recent years. One of the major concepts developed by multiple lines of investigation posits that there is a period in the development of seropositive RA during which there is elevation of autoantibodies, including RF and ACPAs, several years prior to a diagnosis of RA. This autoantibody production occurs in the absence of synovitis (as determined either by physical examination, imaging or, in some cases, synovial biopsy) [1–13].

While the specific aetiology of RA remains elusive, this preclinical period of RA development implies that the disease is initiated at a site outside of the joints. While the exact location is unknown, established and emerging data discussed below support the central hypothesis for our current studies, namely that events leading to RA autoimmunity might originate at a mucosal site.

In this review we will discuss this hypothesis by first succinctly describing the structure, development and function of mucosal surfaces. In particular we will examine the role of microbes in shaping mucosal and systemic immune responses. We will also outline how microbes, as well as other factors such as smoking, can trigger immune responses at mucosal sites and eventually lead to RA. Finally, we propose a research agenda for greater
understanding of the role of mucosal immune responses in the initiation of autoimmunity and RA.

**Mucosal structure and function**

There are a variety of mucosal sites in humans, including the eye, respiratory tract, gastrointestinal tract and genitourinary tract, as well as mammary glands and serosal sites such as the pleural and peritoneal cavities [14–16]. In addition, there are multiple subsites with unique immunological characteristics. As examples, the oral cavity has salivary glands as well as subgingival spaces; the respiratory tract can be divided into the nasopharynx (and even the middle ear), the airways (including the trachea and bronchi) and the aeroeal spaces [17].

In general, the mucosa consists of epithelial cells that form a surface barrier, with hair (in some areas) and a coating of mucus contributing to that barrier (Fig. 1). In some sites, such as the larger airways in the lung, these epithelial cells have cilia that contribute to the removal of foreign material. On the luminal surface there is a variety of components of the immune system, including immunoglobulins, complement and cells that include neutrophils, macrophages, dendritic cells and T and B cells [15, 18–23]. Intermixed with the mucosal epithelial cells are cells that produce mucus (e.g. goblet cells), cells with endocrine function (e.g. enteroendocrine cells in the intestine), cells that participate in a non-specific fashion in host defence (e.g. Paneth cells in the gut) and cells that participate in antigen recognition and presentation, including dendritic cells and M cells. In addition, there are intraepithelial lymphocytes that serve to maintain mucosal homeostasis and are similar to T cells [24]. Underlying the epithelial cell layer in the lamina propria are blood vessels and lymphatics as well as cells that include neutrophils, mast cells, macrophages, dendritic cells, T cells (including effector T cells producing IL-17, Th17 cells and Treg), B cells, as well as NK cells [25].

The mucosal surfaces sit at the interface between the environment and the host and have multiple means that involve both innate and adaptive factors to manage not only potential threats from the environment, such as toxins and pathogens, but also factors that may be beneficial to the organism, such as commensal bacteria. Among the innate factors, lysozyme, lactoferrin, complement, immunoglobulin and cellular elements such as neutrophils and macrophages play important roles. In addition, areas of submucosal organized lymphatic tissue called mucosa-associated lymphoid tissue (MALT) [20, 26] are present in most mucosal surfaces. In the gut, MALT and in particular Peyer’s patches form in utero and with influence from endogenous factors [14]; in contrast, MALT tissue in the nasopharynx begins development only after the tissue is exposed to exogenous flora [27]. In well-developed MALT, cells such as M cells, dendritic cells and macrophages can sample antigens and lead to immune responses. In the lung, an ectopic lymphatic tissue called bronchus-associated lymphatic tissue can form, with local production of antibodies and class switching that can aid in clearance of local insults, apparently only in the presence of inflammation or as a consequence of microbial pathogens [28–30]. Immune responses generated in MALT and ectopic lymphoid structures can then traffic first to regional lymphatics, then systemically and finally back through the circulation to mucosal sites (such as the gut lamina propria) where they can perform effector functions [17, 19]. In particular, several molecules including α4β7 integrin are known to facilitate effector cell homing to the gut mucosa [31]. However, little is known about the specific factors that may induce effector cell homing in other tissues, although these factors likely exist [31].

Immunoglobulins are central players in mucosal immunity. All of the immunoglobulin isotypes (IgA, IgD, IgE, IgG and IgM) may be present at mucosal surfaces [19]; however, the hallmark of mucosal immune responses is the presence of IgA, which is typically in its secretory form (sIgA). IgG is also present at mucosal sites, and can arrive by active transport typically through the neonatal Fc receptor, diffusion from the circulation or local production [17]. IgM is also present at mucosal surfaces, typically in its secretory form. IgD may also play an important role in mucosal responses, including a role in basophil activation and cytokine secretion (IL-4, IL-13) and in particular is present in secretions from the upper airway and nares and in human breast milk [17].

Overall, mucosal immunological structure and function allow for protection against invasion of harmful factors through both mechanical barriers and immune responses. In addition, the mucosa contributes to the generation of beneficial immune responses of protective immunity to many natural infections, allowing the use of oral vaccines, enteric viruses and pathogens and of a nasal vaccine against influenza [32]. However, immune responses that initiate at mucosal surfaces can also lead to harm and in the next sections we discuss in detail how the mucosa balances defence with homeostasis and cooperation with common environmental factors, including the microbiome, and how these relationships may go awry and lead to autoimmunity.

**Microbiome physiology in mucosal sites**

The microbiome, as defined by Joshua Lederberg, is composed of the totality of the ecological communities of symbiotic, commensal and pathogenic microorganisms (and their genomes) that literally share our body space [33]. It has been estimated that about 100 trillion microorganisms live in and on our body spaces and surfaces, outnumbering human cells by a factor of 10 and total protein-coding genes by a factor of 100. Importantly, each mucosal site harbours its own set of distinct microbial communities that exist in the unique mucosal environments.

This characterization of the human microbiome in health and disease states has been catapulted by advances in bacterial DNA-sequencing technologies [34]. In fact, fewer than 20% of bacterial species can be cultured using classical microbiological approaches. Largely due to efforts such as those of the National Institutes of Health Human Microbiome Project [35] and the European Metagenomics of the Human Intestinal Tract consortium, an almost
FIG. 1 Overview of the immune response in the tonsil and regional lymph node as an example of the mechanisms of immunity at mucosal sites

Tonsils, adenoid tissue and the intestine contain M cells that mediate antigen uptake into the tissue rich with lymphoid follicles (A) in which the primary expansion of naive B cells occurs (dendritic cells can also uptake antigen at mucosal sites through processes that extend into the lumen). Antigen presentation is followed by the subsequent generation of memory B cells that populate other lymphoid tissues, especially regional lymph nodes. Dark and light zones containing centroblasts and centrocytes and the mantle zone, in which dendritic cells, B cells and T cells collaborate for B cell activation, are shown in the upper right of (B). A similar expansion of memory (and naive) B cells can occur with secondary exposure in the lymph nodes that are draining the airways as well as other mucosal sites. FDC: follicular dendritic cells; HEV: high endothelial venule. Figure reprinted from Kato A et al., B-lymphocyte lineage cells and the respiratory system. J Allergy and Clinical Immunol 2013;131:933-57 [17], with permission from Elsevier.
complete catalogue of oral, airways, intestinal and skin microbial communities is now available. This characterization of bacterial communities and its biological relationship to mucosal immunology responses have led to new advances in our understanding of their role in health and disease [36]. It has also opened new fields of research suggesting that the microbiome could potentially serve as an environmental factor leading to autoimmunity and related clinical manifestations, as demonstrated by several studies in IBD, psoriasis and inflammatory arthritis [37–39].

For the most part, however, our microbiome fulfills complementary physiological functions vital for our survival, including assisting with metabolic activity and nutrition. In addition, as discussed in more detail below, the microbiome is fundamental for the development of the mucosal immune system and defense against luminal pathogens.

### Development of the mucosal immune system: relationship with the microbiome

Although humans undergo embryogenesis under sterile conditions, immediately postpartum the newborn's body is populated by a range of microbes originating in the surrounding environment. Thereafter, and for life, this vast and dynamic community of microorganisms coexists with us in a complex but mutually beneficial relationship. Initially, however, a period of floral instability is the norm, particularly in the gastrointestinal tract [40]. Slowly, and during the first year of life, both the taxonomic richness and diversity of bacterial species increase. When solid foods are introduced, the gut microbiota expands, becomes more stable and begins to mimic the characteristics of the adult communities [41]. The neonatal period is critical in the establishment of the microbiome and its relationship with the host. Microbial colonization of the intestinal lumen and other sites has a profound effect on the development and function of the immune system. Animals kept under germ-free conditions have impaired development of the mucosal immune system, including lymphoid tissue genesis and organization of Peyer’s patches and lymphoid follicles, secretion of antimicrobial and bactericidal peptides by epithelial cells and mucosal accumulation of immune cells. Immunoglobulins (sIgA) delivered by breastfeeding prevent the translocation of aerobic bacteria from the neonatal gut into draining lymph nodes and results in a protective pattern of intestinal epithelial cell gene expression in adult mice [42]. Throughout adult life, the microbiota continues to affect the host immune system utilizing multiple signal mechanisms, including microbial components and their metabolites. In turn, the immune system is capable of recognizing these factors by activating innate immune receptors. The armamentarium used to prevent tissue damage and antigen translocation includes cellular repairing factors, antimicrobial proteins and secretory sIgA [43–45].

Mucosal microbiota influences innate immune recognition and leads to the development of a diverse and specific mucosal lymphocyte repertoire [46]. Recent paradigm-shifting studies using gnotobiotic experiments have demonstrated how individual components of the microbiota can induce specific populations of immune cells and alter the balance between pro-inflammatory and Tregs at mucosal sites and in the periphery. This has challenged the assumption that microbes and/or their components are only able to activate the innate immune system. The gut commensal segmented filamentous bacterium (SFB), for example, is sufficient to activate Th17 cells in the lamina propria [47] and eventually trigger autoimmunity and inflammatory arthritis [48] (see section Environmental and genetic factors contributing to the generation of autoimmunity at mucosal sites below). One plausible explanation derives from a recent study showing that the TCR repertoire of intestinal Th17 cells in SFB-colonized mice is highly specific and that most Th17 cells, but not other T cells, recognize antigens encoded by SFB. This explains potential mechanisms of Th17 cell induction by microbiota and how gut-induced Th17 cells can contribute to distal organ-specific autoimmunity [49]. For their part, *Bacteroides* species and their molecule polysaccharide A are unique in that they appear to be specific inducers of Tregs in the mucosa [50] and provide protection against the development of IBD [51] and multiple sclerosis in an animal model [52].

In humans, microbiota composition is influenced by diet, antibiotic use, infections and possibly the host genome. When this fine equilibrium is altered in an unfavourable way, a state of dysbiosis ensues, typically characterized by an overgrowth of potentially pathogenic bacteria (termed pathobionts) and/or a decrease in the number of beneficial bacteria [53, 54]. A growing body of evidence has shown a correlation between dysbiosis, autoimmunity and systemic inflammation. This is true for both animal models and human studies and has been reported in various conditions, including not only IBD [55, 56], but also systemic autoimmune diseases such as type 1 diabetes [57], encephalomyelitis [58] and RA [48, 59, 60]. The following section provides detailed evidence for the implication of the microbiome in local and systemic autoimmune processes.

### Environmental and genetic factors contributing to the generation of autoimmunity at mucosal sites

Several indirect lines of evidence support mucosal surfaces as sites of generation of RA-related autoimmunity. In particular, Barra et al. [61] demonstrated that the proportion of IgA ACPAs was higher than IgG in subjects at risk for future RA; intriguingly, in patients with established RA, the proportion of IgG ACPAs was higher than IgA. This finding supports the notion that early RA-related autoimmunity may be triggered by mucosal processes generating IgA-related autoimmunity, later transitioning into an IgG response that eventually leads to clinical manifestations of disease (i.e. synovitis). There have also been associations (albeit controversial) between serological evidence for certain infections, including *Proteus* and...
MALT: mucosa-associated lymphoid tissue.

**Table 1** Mechanisms by which the mucosa may be involved in the development of autoimmunity

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**Mycoplasma** species, and risk for RA [62–64]. In addition, Mikuls et al. [65] demonstrated an association between RA-related autoantibodies in healthy, first-degree relatives of probands with RA and serum antibody titres against *Porphyromonas gingivalis*—an organism implicated in human periodontal disease (PD)—suggesting that immune responses to *P. gingivalis* may play a role in early RA-related autoimmunity.

There are several mechanisms by which the mucosa might contribute to the development of RA-associated autoimmunity (Table 1). Cross-reactivity may occur between mucosal antigens (which may be microbial in origin) and self-proteins, a process thought to occur in rheumatic fever, where pharyngeal infection with certain species of *Streptococcus* leads to the generation of antibodies that cross-react with self-antigens in the heart and other tissues [66]. In addition, mucosal generation of neo-antigens (e.g. citrullination) by pathogen-mediated inflammation and peptidylarginine deimination activation, or carbamylation, could occur at mucosal sites through microbial-related respiratory burst [67–69].

In RA, the finding of local enrichment of ACPA in both bronchoalveolar fluid of early untreated RA patients and in induced sputum of arthritis-free individuals at risk of developing RA [70] (in some cases, even in the absence of the same antibodies in the blood) supports the notion that autoimmunity might indeed originate at mucosal sites. In line with this, tissue inflammation and ectopic lymphoid structures were described in bronchial biopsies of patients with early untreated ACPA-positive RA in the absence of any other associated lung disease [71]. This was also shown in lung biopsies of ACPA-positive individuals with chronic lung disease but no signs of joint inflammation [72]. Interestingly, similar structures containing citrullinated protein-binding B cells have also been reported in patients with established RA and associated lung disease [73]. Signs of lung inflammation have further been described by high-resolution CT imaging of both ACPA-positive healthy individuals at high risk for RA development, even in the absence of smoking [8], and patients with early untreated ACPA-positive RA [74]. An alternative explanation for ectopic lymphoid tissue formation is a secondary immune injury of the lungs induced by ACPA in the presence of citrullinated proteins in the lungs. However, these two different scenarios are complementary and not mutually exclusive.

In additional support of a mucosal-based trigger for RA, exposure to tobacco smoke is the strongest environmental risk factor associated with RA, with some estimates that smoking explains ~30% of the risk for ACPA-positive RA [75]. However, despite this association, the role of smoking in disease pathogenesis is still uncertain. Original studies showed that smoking increases the expression of citrullinated proteins in the lungs of healthy smokers [76]. This was later confirmed for early untreated RA patients as well [74]. Interestingly, increased expression of citrullinated proteins was present not only in smokers, but also in ACPA-positive non-smokers, suggesting that factors other than smoking might also contribute to the generation of citrullinated epitopes in the lungs.

The presence of susceptibility genes (and in particular HLA-DR SE) in smokers further increases the risk of developing seropositive RA, as demonstrated by several large epidemiological investigations [77–83]. This might be explained through specific interaction of the susceptibility genes with citrullinated but not native (i.e. arginine) epitopes [84–86]. In addition, peripheral blood B cells of both RA patients and healthy individuals carrying the SE alleles show increased ACPA production when exposed to smoking, suggesting that both environment and genetics influence the pool of autoreactive B cells in healthy subjects [87]. Somewhat controversial data have recently emerged from studies in animal models exposed to chronic cigarette smoking [88]. In these experiments, exposure of HLA-DR4 transgenic mice to cigarette smoking unexpectedly suppressed CIA, while enhancing innate immunity and mounting a robust response to citrullinated vimentin. In contrast, similar exposure in HLA-DQ8 transgenic mice (occurring in linkage with DR4 in humans) not only augmented the antigen-specific adaptive T cell responses to native and citrullinated proteins, but also worsened the course of CIA.

One interpretation of these findings is that DR4 contributes to autoimmunity by enhancing innate immune responses potentially following bacterial challenge, while DQ8 might contribute to antigen-specific autoreactive processes. However, the arthritis-suppressive effect of smoking in HLA-DR transgenic mice and the relevance...
of these findings in human disease remain to be elucidated.

**Microbial factors contributing to the generation of autoimmunity at mucosal sites and their interaction with environmental and genetic factors**

As mentioned above, inconclusive studies have implicated infections with specific organisms in the pathogenesis of RA [62, 63, 89, 90]. The emerging complexity of the human microbiome, coupled with new methods for culture-independent microbial DNA sequencing, today allows studies on how the microbiome interacts with genetic and environmental factors and contributes to disease [60, 91].

Microbiome composition is partially determined by the host genome, as first suggested by early studies in twins [92, 93], although some conflicting observations also exist [94, 95]. Subsequent studies in congenic mouse strains have revealed that MHC genes might have a prominent effect in determining the composition of the gastrointestinal microbiota [96]. While these observations do not necessarily imply the same genetic associations as for RA, studies in HLA-DR transgenic mice have shown differences in the relative abundances of gut microbiota between arthritis-susceptible HLA-DRB1 0401 and arthritis-resistant HLA-DRB1 0402 transgenic mice [97]. Moreover, germ-free conditions lead to abrogation of spontaneous arthritis in IL-1 receptor antagonist knockout mice [59] and attenuation of spontaneous arthritis in K/BxN TCR transgenic mice. Interestingly, the wild-type animals do not develop arthritis, even in the presence of pro-arthritogenic gut flora [48]. It has also been shown that new-onset untreated seropositive RA patients have an overexpansion of gut Prevotella copri [60], a recently described species with connection to IBD and atherosclerosis [98]. Curiously, RA patients with a greater abundance of *P. copri* are mostly those with negative SE alleles.

Beyond the gut, the oral and respiratory tract microbiome, and in particular changes in the oral microbiome related to PD, have long been implicated in the pathogenesis of RA. It is well known that PD shares multiple risk factors with RA, including smoking and the genetic association with PD, but the exact causality or association between these two disease states is not yet completely understood [99]. Interestingly, α-enolase of both bacterial (*P. gingivalis* derived) and human origin was immunogenic in HLA-DR transgenic mice, with generation of antibodies recognizing both native and citrullinated forms of human enolase. However, an arthritogenic effect could not be replicated. A possible explanation for this discrepancy may be attributed to local environmental conditions, in particular the pathogen status in different specific pathogen-free facilities [100, 101].

Importantly, despite data documenting a relevant role for the lower respiratory tract as a mucosal initiating site of RA-associated autoimmunity [70, 74, 102], no study to investigate the lung microbiome in RA is currently available. Preliminary data suggest that the lung microbiome is different in asymptomatic subjects with elevated risk of future RA when compared with healthy controls [103]. It should be noted that environmental factors—and smoking in particular—have a large effect on the mucosal microbiome composition [104–111]. These are issues that will need to be explored in future studies.

**Expansion of localized autoimmunity to joints and distal sites**

As mentioned above, antibodies are released in the peripheral blood and circulated in the body for years before any clinical sign of joint inflammation [5–7, 112–115]. This strongly suggests that antibodies are generated at extraarticular sites, but does not completely exclude the possibility that they might still be produced in the joints in the absence of any macroscopic and/or microscopic signs of inflammation. These healthy individuals lack not only clinical complaints, but also any clear-cut sign of joint inflammation [9, 116], raising the possibility that antibodies are passive bystanders of the disease. However, ACPAs are able to exacerbate existing minimal joint disease by passive transfer in mice [117] and possess several effector pathogenic functions. ACPAs activate the complement system [118] and promote macrophage activation when incorporated in immune complexes via either Fcγ receptors or TLR-4-dependent mechanisms [119]. More recently, antibodies against mutated citrullinated vimentin were shown to promote bone resorption *in vitro* and to induce osteoclastogenesis by adoptive transfer into mice [120]. ACPAs can also stimulate neutrophils to release neutrophil-derived extracellular traps and promote inflammation [121] (Fig. 2).

Despite these advances in understanding how ACPAs might contribute to perpetuation of joint inflammation, it remains unclear what event or series of events is required for the delayed initiation of this joint inflammation, although there are several possibilities. First, it is plausible that a yet unidentified second hit (such as minor trauma or transient infection/microbiota community alteration) could lead to expression of citrullinated proteins in an otherwise citrullinated protein-poor healthy joint [67] and that these antigens would then be targeted by the pre-existing circulating autoantibodies, ultimately leading to clinical signs of joint inflammation. This would imply that circulating autoantibodies that may have been generated in response to mucosal antigens would target similar antigens in the joint. Supporting this, it has recently been shown that the same citrullinated peptides could be identified by mass spectrometry in the lungs and joints of RA patients [122]. Second, it is possible that sites other than the synovial membrane are the first joint component to be affected, with secondary synovial involvement being an epiphenomenon. In line with this, recent studies utilizing microcomputer tomography showed that signs of bone destruction are present before clinical onset of synovial inflammation [121]. Third, progressive epitope spreading...
and the emergence of subclinical inflammation (as seen for certain cytokines and chemokines) [124, 125] might be needed to alter the number and/or specificity profile required by ACPAs to gain these effector functions. In this context, it is worth mentioning that all available evidence showing direct pro-arthritogenic properties of ACPAs have been obtained using antibodies purified from peripheral blood and/or SF of patients with already established disease. This leaves open the possibility that antibodies might emerge from mucosal interactions first as non-pathogenic immunoglobulins during the preclinical phase only to gain arthritogenic properties through epitope spreading or pathogenic changes in avidity, affinity or Fc function at a later time point. These changes could potentially allow ACPAs to target the joints and cause inflammation. These issues remain to be addressed in high-quality natural history studies of RA that can include broad evaluations of innate and adaptive immunity at mucosal, systemic and joint sites.

Summary and future directions

As discussed above, several aspects of the mucosal system biology, including its relationship to a variety of commensal as well as pathogenic organisms, make it an attractive frontier in rheumatological research. However, several challenges on how to explore this frontier are still elusive (Table 2). Perhaps the most important factor in advancing the understanding of the role of mucosal biology in the development of rheumatic disease will be the careful utilization of well-characterized human cohorts followed longitudinally in various phases of development of rheumatic disease. These should range from a healthy state to preclinical disease (i.e. where there is evidence...
TABLE 2 Research agenda for the study of mucosal biology in relation to rheumatic diseases

<table>
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<tr>
<th>High-quality longitudinal natural history studies of rheumatic disease that can be used to evaluate the temporal relationship between mucosal inflammation, exposure to environmental risk factors (including the microbiome) and genetics on the development of autoimmunity.</th>
<th>2 Aho K, Heliovaara M, Knekt P et al. Serum immunoglobulins and the risk of rheumatoid arthritis. Ann Rheum Dis 1997;56:351–6.</th>
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<td>Robust, safe and feasible methods to assess mucosal biology in human subjects. Methods to reliably obtain high-quality biospecimens that can be tested for a variety of factors, including metabolic, immune and microbiome, need to be established for each mucosal region.</td>
<td>3 Aho K, Palosuo T, Heliovaara M. Predictive significance of rheumatoid factor. J Rheumatol 1995;22:2186–7.</td>
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<td>Robust analytical methods to evaluate the relationship between the microbiome and mucosal inflammation and autoimmunity. These methods should include means to assess microbial relationships between sites (e.g. oral and lung, gut and genitourinary) and allow for robust assessment of changes over time.</td>
<td>4 del Puente A, Knowler WC, Pettitt DJ, Bennett PH. The incidence of rheumatoid arthritis is predicted by rheumatoid factor titer in a longitudinal population study. Arthritis Rheum 1988;31:1239–44.</td>
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<td>Identification of specific mechanisms by which autoimmunity is generated at a mucosal site (molecular mimicry, alteration of human proteins or creation of an inflammatory environment in which autoimmunity develops).</td>
<td>5 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;48:2741–9.</td>
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<td>Identification of mechanisms by which a mucosal surface (such as the lungs) may be a site of initiation of autoimmunity as well as a target of immune-mediated inflammation and damage.</td>
<td>9 van de Stadt LA, Bos WH, Meursinge Reynders M et al. Duration of preclinical rheumatoid arthritis-related autoantibody positivity increases in subjects with older age at time of disease diagnosis. Ann Rheum Dis 2012;64:1756–61.</td>
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