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Insights from Populations At-Risk for the Future Development of Classified Rheumatoid Arthritis

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SYNOPSIS

Rheumatoid arthritis (RA) is characterized by the development of disease through a progressive series of stages. In the seropositive subset of patients with classified RA, a preclinical stage is present for a substantial number of years prior to the onset of clinically apparent inflammatory arthritis. Multiple environmental, hormonal and lifestyle factors likely play important roles in the initiation of RA-related autoimmunity during this very early period and the transition to clinically apparent arthritis. This preclinical period is characterized by the presence of autoimmune and inflammatory processes that can be assessed and quantitatively measured using biomarkers. In conjunction with qualitative phenotypes, these data can be used to develop algorithms designed to provide highly predictive estimates for the future onset of classifiable disease. Relevant biomarkers and measures for this period include autoantibodies, alterations of antigen-specific and innate lymphoid populations, elevated cytokines/chemokines, genetic/genomic factors, imaging study results, associated clinical findings, dietary and environmental biomarkers, assessments of cardiovascular disease and other comorbidity risks, microbiome analyses, and metabolomic changes. Most prior studies have used retrospective analyses of serum bank samples to study this period of disease evolution. A major challenge, however, has been how to identify from the contemporary population of asymptomatic subjects who are at sufficiently high risk for future disease to be informative and representative of “preclinical patients”. To find such subjects, studies have been performed using first-degree relatives of patients with RA as well as other populations who present to physicians in health care settings. This chapter will review the results of analyses that have been undertaken in these “at-risk” subjects, summarize the evidence which suggests that such populations are relevant to understand disease pathogenesis, and illustrate how the results of these studies have informed our current views of the initiation and early evolution of RA prior to the onset of clinically apparent disease.

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Dr. Holers receives royalty payments for a licensed patent for the use of biomarker patterns in the diagnosis of rheumatoid arthritis.

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Preclinical Rheumatoid Arthritis; Epidemiology; Biomarkers; Autoantibodies; Dietary Factors; Prediction; Prevention; Imaging

INTRODUCTION

Stages in the Evolution of RA

Rheumatoid arthritis (RA) encompasses two major subsets of disease, seropositive and seronegative (1;2). Seropositive individuals exhibit RA-related autoantibodies, which include antibodies to citrullinated protein antigens (ACPAs), with this posttranslational modification most commonly found in an antigenic form on fibrinogen, vimentin, type II collagen and enolase (3;4). In addition, antibodies to the Fc domains of self Ig molecules that are designated rheumatoid factors (RF) are also found (5). Based on clinical comparisons as well as studies of environmental and genetic associations, it is considered that seropositive and seronegative forms of RA likely exhibit overlapping but distinct pathogenic mechanisms (6;7).

The current understanding of the natural history of seropositive RA is summarized in Figure 1. In this regard, one might consider that the onset of RA occurs around the time that clinically apparent arthritis appears. However, there is a prolonged period characterized by the presence of highly specific RA-related autoimmunity, including both ACPA and RF, in patients that typically begins 3–5 years (8–16) prior to the onset of clinically apparent disease [reviewed in (17)]. Although this period is defined retrospectively as the “preclinical” period of RA in subjects who eventually develop the disease, it is perhaps best designated an “ACPA and/or RF+ at-risk” status in the populations being studied in cross-sectional or prospective studies, as the eventual outcome in individual subjects is yet unknown (18). Subjects may progressively develop further immune alterations and then clinically apparent arthritis. It is considered possible to “reverse” the disease course at these early points (18;19), although it is uncertain as to the proportion of subjects who do so and the primary determinants of such a change.

Herein evidence is presented addressing the question of whether intensive studies and deep phenotyping of individuals in the ACPA+ and/or RA-related autoantibody positive at-risk status can inform the field with regard to the mechanisms by which the earliest immunologic abnormalities develop and what biologic processes may be the early “drivers” of disease. As outlined below, the results in aggregate of these studies of at-risk individuals do provide such insights and strongly suggest that the initiation RA likely involves an extra-articular, and most likely a mucosal, inflammatory process and/or dysbiosis.

Early Stage Studies of RA Natural History Utilize Several Approaches to Define the “At Risk” Population

At-risk populations have been defined based on several characteristics, with the most commonly utilized being a close familial relationship, usually at the first-degree relative (FDR) level. FDRs are studied because these individuals exhibit a 3–5 fold increase in

lifetime risk for the development of classified RA (20;21). Additionally, though, at-risk individuals can be identified by the carriage of the shared epitope (SE)-containing HLA alleles which are overrepresented within RA populations (22;23). A third approach is the identification of subjects who present to health care settings with RA-related autoantibodies and non-specific musculoskeletal symptoms without arthritis, designated as an “arthralgia” population (24–26). Finally, at-risk subjects can be identified through large health fair screening primarily designed to identify clinically active but undiagnosed RA, and wherein RA-related autoantibody positive subjects without arthritis or signs/symptoms of classified RA are also found (27).

For practical reasons relating to the lack of ability to accurately classify individuals who do not elaborate RA-related autoantibodies, many studies of at-risk individuals have largely focused on the RA-related autoantibody positive population. However, because there are also immune abnormalities associated with the autoantibody negative FDR population (22;28), as well as modest increases in cytokines and chemokines as the onset of clinically apparent seronegative disease approaches (8), utilizing a more inclusive definition of the at-risk population is also useful for studies.

Presence of Biomarkers in the Preclinical Period and Implications for Disease Pathogenesis

1. Autoantibodies

Findings in Classified RA: ACPA and RF are commonly found in RA and indeed are components of the definition of seropositive disease (29;30). As a practical matter, ACPA are typically measured using commercial ELISA-based tests that are certified and approved for clinical use, and when assessed using these methods are designated anti-cyclic citrullinated peptide (anti-CCP) antibodies (31;32). Anti-CCP antibodies have been described in subjects with classified RA as demonstrating a sensitivity of 60–70% with a specificity of ~98% (3;33), improving substantially over the ~80–85% specificity of RFs (5). However, the specificity of ACPAs differs by the specific anti-CCP commercial test utilized, the control population against which the test is compared, and the stage of disease being evaluated (34). In addition to ELISA tests, there are an increasing number of peptide- and protein-based array methods that can be used to refine the epitope specificity of ACPAs, which demonstrate a very wide variety of non-cross-reactive citrullinated peptide specificities in patients with classified RA (35;36).

In addition to ACPA and RFs, several other autoantigens are recognized by smaller subgroups of patients with classified RA. These autoantibodies have been reported to recognize carbamylated proteins antigens (anti-CarP) wherein arginine is converted to homocitrulline (37), mutated citrullinated vimentin (MCV) (38), RA-33 (39), and peptidyl arginine deiminase type 4 (PAD-4) (40), among others.

Findings in At-Risk Populations: Using stored serum banks to study the evolution of autoantibodies in the preclinical period, both increase in titers and epitope spreading have been found to occur that encompasses a wider variety of targets as the clinical onset nears (8;35;41). With regard to assessment of subjects in real time, a subset of FDR elaborate RA-

related autoantibodies, analyzed as anti-CCP2, anti-CCP3.1, RF by nephelometry, and RF by isotype positivity. When analyzed, a largely non-Hispanic White FDR population was found to exhibit an ~16% positivity inclusive of this range of autoantibodies [(23), and updated], and the North American Native population was reported to exhibit anti-CCP antibodies in 17% of FDR, 11% of more distant relatives, and 3% in controls (42). In FDR within multi-case families in Sweden, concentrations and frequencies of anti-CCP and RF isotypes were also significantly increased. Of note, in that population the distribution of IgA and IgM isotypes was higher than IgG in the relatives, whereas the IgG isotype dominated in probands with RA (43).

FDRs have also been studied to determine whether there are similar increases in epitope spreading associated with an anti-CCP positive test. One study showed that anti-CCP positive FDRs elaborate a substantially increased number of ACPA than control FDRs without RA-related autoantibodies, with a subset being positive for 9 ACPA in a pattern typical for classified RA (44)]. An increasing number of positive ACPAs was also associated with the presence of a tender joint on exam. In another independent study of an FDR population using a six-member ACPA panel, a high prevalence of ACPAs (48%) and was found as compared to controls (10%), with citrullinated vimentin most commonly targeted (45). Of note, a high proportion of ACPAs in that study were of the IgA isotype.

Thus, RA-free FDRs show reactivity to multiple ACPA, demonstrating that both an anti-CCP positivity in this population is not likely to be a “false positive” but rather is similar in nature to subjects with classified RA, and that there is evidence of genetic and/or familial effects within the FDR population. What is yet unknown is whether other anti-CCP characteristics found in patients with classified RA, such as increased avidity (46), substantial complement activating potential (47), and altered G0 carbohydrates that promote IgG effector functions (48;49) are also present in samples from these real time at-risk populations, although these questions are under study. Additionally, as only 3–5% of FDRs will ultimately develop RA, the high proportion of immune abnormalities in FDRs must either not be a stable phenotype, reflect early immune dysregulation that progresses to other autoimmune diseases in this population, or that the individuals elaborating the immune dysregulation do not all progress through additional stages of disease. Although limited in scope, evidence for both conclusions is present. Finally, reflecting clinical variability in early arthritis, in subjects enrolled in the Canadian Early Arthritis Cohort who underwent baseline and at least 12-month follow up studies, both RF and ACPA were found to fluctuate, with ~10% of subjects converting from ACPA– to ACPA+, and vice versa.(50).

2. Alterations of Antigen-Specific and Innate Lymphocyte Populations

Findings in Classified RA: CD4 T cells specific for citrullinated synovial antigens have been identified and quantitated through the use of MHC class II tetramers, where samples from patients with classified RA display an increased frequency of citrullinated peptide (cit)-specific T cells, predominantly of the memory Th1 cell class (51). Notably, the frequency of cit-specific T cells in RA patients is highest within the first 5 years of disease, and is decreased among patients on biologic therapies independent of disease duration. Autoreactive T cells in patients with RA recognize a combination of citrullinated epitopes

carried on class II tetramers (51), as well as demonstrate evidence of cellular immunity to citrullinated autoantigens using other techniques. For example, secretion of IL-6, IL-17 and tumor necrosis factor (TNF)-alpha by CD4+ cells was found to be present in normal controls and patients with RA, while interferon (IFN) and IL10 were specific for RA, with citrullinated aggrecan generating the most robust antigen-specific response (52).

With regard to B cells, subjects with RA exhibit an increased number of peripheral blood plasmablasts and/or plasma cells, a substantial proportion of which produce ACPAs (53). Plasma cells producing ACPAs are also commonly found in the synovium (54). B cells in patients with active RA also exhibit features of dysregulated control of anergy checkpoints during development (55;56), as well as signaling alterations in the anergic population (57).

In addition to antigen-specific lymphocytes, innate lymphoid cells (ILC), a novel family of effector cells that are reported to play important roles in the maintenance of lymphoid tissue, tissue repair and response to infection, are also altered in patients with RA (58). In this regard, there are several distinct subsets of ILC that secrete a variety of cytokines that are important in RA pathogenesis (IL-5, IL-13, IL-17, IL-22, TNF-alpha and IFN-gamma).

Findings in At-Risk Populations: Although evidence of dysregulation of autoimmune B and T cells, as well as ILCs, is anticipated to be present in the at-risk population, the studies necessary to assess this question are only just underway. In one sense, because antigen-specific T cells are more likely to be found earlier rather than later in disease (51), systemic high titers of anti-CCP antibodies can also be present in FDRs (42–44), and local production of anti-CCP antibodies has been described in the at-risk populations (28), it is very likely that antigen-specific lymphocytes will be present and detectable. However, major questions include the epitope specificities of this early response, whether there a linked B and T cell response that is present, and if there are peptide specificities that can be targeted using antigen-specific immune tolerization approaches.

3. Cytokines and Chemokines

Findings in Classified RA: Patients with classified RA exhibit a wide variety of elevated cytokines, with no single cytokine or pattern predominating [see for example, (59)]. There are patterns, though, that are associated with a highly inflammatory state, and IL-1, TNF-alpha, IL-12p40 and IL-13 have been found to be particularly elevated in early RA (59).

Findings in At-Risk Populations: Using retrospective serum studies, it has been found that shortly before the onset of clinically apparent arthritis, systemic levels of cytokines/chemokines increase in a very heterogenous manner, both with regard to the number being elevated in an individual subject and the levels of each factor (8;16;60;61). With regard to at-risk populations, elevated cytokines are associated with the presence of anti-CCP2 antibody (62), as well as expanded in FDRs regardless of autoantibody status (22). Thus, there is substantial evidence that immune dysregulation in at-risk populations is associated with systemic evidence of inflammation. Prospective analyses of this question in at-risk populations, however, have not been reported, and studies of other inflammation-related biomarkers including complement activation fragments are also now underway.

4. Genetics

Findings in Classified RA: There are many genes which associate with RA, including high risk HLA-DR alleles containing the SE (63) and *PTPN22* (64), as well as over 100 other linked genes with a modestly elevated relative risk (65). Notably, a substantial number of these genes encode products are components of immune-related signaling pathways [reviewed in (66)] and/or also encompass genes expressed in memory effector T cells (67) and associated with CD40, TRAF1, TNFAIP3 and PRKCQ pathways (68).

Differences in genetic relationships disease are also present that are based on environmental exposures, such as a relationship between smoking, SE and the presence of ACPA (7;69), and linkage between SE and the presence of antibodies to citrullinated enolase (70).

Findings in At-Risk Populations: Despite the increasingly understood relationships between genetic risk and the presence of classified RA, little has been accomplished yet regarding the question of at what point during disease evolutions these factors act. With regard to the major risks associated with the SE, in one study there was a trend between the presence of ACPA and the SE, but the size of the study apparently limited the ability to definitively answer this question (45). Thus, this is another question that should be addressed and is indeed under active analysis.

5. Genomics

Findings in Classified RA: In addition to the hard-wired genetic contributions, there are genomic and epigenetic changes that are associated with RA and affect lymphocytes, fibroblast like synoviocytes (FLS) and other cell populations (65;71). Epigenetic modifications include DNA methylation, histone methylation, histone acetylation, histone phosphorylation, and expression of microRNAs (miRNAs), and changes are especially prominent in FLS, where they are chronically exposed to an environment rich in pro-inflammatory cytokines and mediators of oxidative stress (71;72). These epigenetic changes represent key means by which environmental factors could influence gene expression and disease heterogeneity, and potentially heritability given that some epigenetic changes can be transmitted to offspring (71;72). One important finding is that epigenetic changes in FLS appear to be “imprinted” and durable even after removal from the pro-inflammatory environment (71).

Findings in At-Risk Populations: It is not yet known whether the same epigenetic findings in classified RA are also found in the preclinical disease state, or whether other stage-specific changes are found. One particularly important question is whether FLS exhibit changes that promote the transition from the presence of circulating autoimmunity to the development of local synovitis.

6. Imaging and biopsy studies

Findings in Classified RA: Many types of imaging approaches have been applied to the study of patients with classified RA. Perhaps most relevant to the study of preclinical RA and at-risk populations, though, where the question of the presence or absence of changes consistent with inflammatory arthritis is most pressing, are ultrasound (US) and magnetic

resonance imaging (MRI) [reviewed in (73)]. Using these techniques, one can detect by US synovitis through identifying within the images effusions, synovial expansion and a power Doppler signal. In addition, through US one can detect bony erosions and measure cartilage thickness. In addition, it is useful for tendon assessments and defining tenosynovitis and tendon rupture. MRI can also define the same structures but in addition can visualize bone marrow pathologies not detectable by US, such as bone marrow edema.

Findings in At-Risk Populations: Studies in at-risk populations have focused on RA-related autoantibody positive individuals as well as subjects who present to health care settings with arthralgia or early or unclassified inflammatory arthritis. Studies of anti-CCP positive arthralgia patients without clinically detectable arthritis have demonstrated histologic findings without inflammation but with a trend towards CD3 and CD8 T cell infiltration (74). Studies of FDR using MRI of the hands and feet have not demonstrated evidence of synovial inflammation (25), and analyses of arthralgia/IA populations demonstrate that a subset exhibit US abnormalities (75). Thus, the situation remains uncertain, but is an important area for prospective investigation, as well as for the development of additional functional imaging approaches.

7. Dietary, hormonal, and environmental exposures and biomarkers

Findings in Classified RA: With regard to environmental influences on RA susceptibility, there are several well-accepted exposures which increase or decrease the risk of developing RA, including smoke exposure, hormonal factors, dietary exposures, air pollutants and silica dust (69;76); however, the exact causal relationships are not well understood beyond a potential relationship between smoke exposure and citrullination (6). In this regard, autoreactivity to citrullinated alpha-enolase has demonstrated a linkage to both smoking and DR4 shared epitope status (70). Dietary factors have also been assessed, and particularly compelling findings have included an inverse relationship between omega-3 fatty acid intake and both the risk of developing and severity of RA [reviewed in (77)].

Findings in At-Risk Populations: Although limited in scope, studies have demonstrated an inverse relationship between birth control exposure, as well as a direct relationship to smoking, with the presence of RF in an FDR at-risk population (78). In the same population, no association with Vitamin D levels was found (79). In addition, the relationship between air pollution exposure and RA-related autoantibodies in the FDR population has been studied, and demonstrated no relationship (80). Ongoing studies are evaluating the relationships between omega-3 fatty acid intake and presence of RA-related autoantibodies.

8. Cardiovascular disease risk

Findings in Classified RA: In addition to arthritis, other common clinically significant manifestations of RA include an elevated risk for cardiovascular disease, although the exact time point at which this elevated risk is manifest is still uncertain (81).

Findings in At-Risk Populations: Reported studies of this important question are still limited in nature, and many are ongoing in a number of at-risk populations. Importantly,

identifying a risk of CVD that precedes clinically apparent articular RA may drive interventions not only to prevent future RA, but CVD as well.

9. Osteopenia and Erosions

Findings in Classified RA: Loss of bone as manifest by osteopenia and local erosions commonly occurs the most severe forms of RA, likely through both local and systemic inflammatory and remodeling processes that alter relative rates of bone formation and degradation by osteoclasts and osteoblasts (82;83). ACPA directed to citrullinated vimentin may be particularly important in inducing bone loss in patients with RA (84).

Findings in At-Risk Populations: Studies in at-risk populations are limited; however, ACPA positive individuals without detectable synovitis have been found to demonstrate diminished bone by the use of micro-computed tomography (CT) analyses (85). Thus, it appears that in addition to evidence of systemic inflammation by cytokine elevations, local bone loss may also characterize the RA-related autoantibody at-risk status.

10. Pulmonary Disease

Findings in Classified RA: Many links between RA and the lung have been made. For instance, cigarette smoke exposure is highly associated with risk for the development of RA, especially in the presence of HLA expressing the DR4 SE (6;69). In addition, through the use of sensitive high-resolution computed tomography (HRCT), patients with early RA demonstrate a substantial number of pulmonary abnormalities (86).

Findings in At-Risk Populations: Notably, at-risk individuals who do not have RA but exhibit high-risk RA-related autoantibodies demonstrate a high rate of inflammatory airway disease that is not associated with a smoking history or other similar exposures (87). Local production of ACPA and other RA-related autoantibodies has been demonstrated through studies of induced sputa from FDR, where RF and anti-CCP antibodies can be readily demonstrated in the sputum but not peripheral blood of a subset of this population (28). Because of these findings, the lung is now considered to be a primary site of the initiation of RA, although other mucosal sites may also be candidates for initiation of RA.

11. Microbiome analyses

Findings in Classified RA: Several lines of investigation suggest that dysbiosis occurs in patients with RA, which may be associated with risk, severity or targets of injury. For example, an elevated occurrence of severe periodontitis is reproducibly found in patients with RA, and conversely patients with RA who exhibit severe periodontitis have higher disease activity scores (88). Patients with severe periodontitis also manifest higher titers of IgG and IgM antibodies to *Porphyromonas gingivalis*, which is important because this organism exhibits enzymatic activity that can citrullinate fibrinogen and enolase in the presence of bacterial gingipains (89). In addition, though, other studies have found that *Prevotella* and *Leptotrichia*, but not *Porphyromonas gingivalis*, are present in higher numbers in the subgingival microbiota in patients with recent onset untreated RA (90).

Finally, studies of the gut, a mucosal site where microbes are well known to be able to influence immune responses, and where experimental arthritis has been modulated by the exposure in the gut to a single organism (91), have suggested that change in the microbiota are associated with concurrent RA (92), especially an expansion of *Prevotella copri* species (93).

Findings in At-Risk Populations: There is strong potential for the mucosa and/or mucosal dysbiosis to be an early driver of RA development. The rationale is based on recent studies strongly suggesting that mucosal inflammation (87), production of RA-related autoantibodies (28) and dysbiosis (93) can occur coincidently. In addition, in at risk subjects, there is a specific elevation of antibodies to *Porphyromonas gingivalis* but not to related strains (94). Therefore, substantial efforts are currently being made to further address these relationships in at-risk populations.

12. Metabolomics

Findings in Classified RA: Measures of environmental and genetic influences include metabolic fingerprints that can be assessed through nuclear magnetic resonance (NMR) spectroscopy-based metabolomics techniques. With regard to patients with classified RA, the serum metabolic fingerprint was found to be clearly different than controls, with lactate and lipids as discriminators of the inflammatory burden in early RA, and differences were also related to the level of C-reactive protein (95).

Findings in At-Risk Populations: As a means by which one can assess a sum of many different endogenous and exogenous influences, metabolomics studies hold promise for helping to understand the biologic underpinnings of RA initiation and early evolution. Because of this, there are ongoing studies to evaluate this question, the results of which should be available shortly.

Integration of Studies of At-Risk Individuals into Stages of Disease Development: As is apparent from the status of many of the studies of at-risk populations that are described herein, this is a very active area of investigation. Notably, only a subset of environmental, and likely genetic, influences appear to be involved during the very early stages of development of RA. This is not surprising, given the complexity of this disease, and also that stage-specific influences are found in other autoimmune diseases such as Type 1 diabetes (96). One way to portray the current state of understanding in RA is illustrated in Figure 2. This figure also illustrates how much further the field needs to advance in order to understand, for instance, the stage-specific genetic influences and whether exposures early but not in later transitions. Nevertheless, the results of recently reported and ongoing studies do suggest that important insights into pathogenesis and treatment can be made in at-risk populations and that these can lead to novel population and individualized prevention and therapeutic strategies.

FUTURE CONSIDERATIONS

The results above indicate that a subset of biomarkers commonly found in patients with classified RA are present in subjects who are at an increased future risk for developing

clinically apparent RA. These results are consistent with a scenario wherein chronic mucosal inflammation that is related to genetic, environmental, microbial and/or other local factors promoting immune dysregulation leads to the initial break in tolerance to self-antigens and systemic autoimmunity manifest by both IgG and IgA autoantibodies. Little is known regarding the precise mechanism that enables the transition from an autoimmune and inflammatory reaction at a mucosal site associated with circulating autoantibodies to an attack on the joints. Nevertheless, several potential mechanisms exist and have been explored either in patients or animal models [reviewed in (97;98)]. One possibility, reflecting the presence of circulating immune complexes containing citrullinated fibrinogen in patients with RA (99), which notably can more effectively activate macrophages through the synergistic engagement of TLR4 and Fc receptors (100), is that immune complexes can enter the joint and deposit on cartilage and in synovial tissue. Another possibility is that transient expression of citrullinated antigens allows the ability of circulating autoantibodies (101;102) or T cells (103) to recognize and initiate or amplify inflammation. A third possibility, potentially explaining the link between the early involvement of the lung with later arthritis, may be shared expression of citrullinated vimentin that is recognized by pathogenic autoantibodies at both sites (104). Further cross-sectional and especially prospective studies of at-risk subjects in this period will allow the definitive elucidation of causal mechanisms that will likely allow treatment with strategies that are unique to this early period of development.

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KEY POINTS

- Seropositive rheumatoid arthritis typically begins with a prolonged period that is characterized by circulating disease-specific autoantibodies in the absence of clinically apparent inflammatory arthritis, with this period being termed 'Preclinical RA' in those who ultimately develop classified disease.
- As the point of clinically apparent disease approaches, Preclinical RA is characterized by increasing epitope spreading of new ACPA peptide specificities, elevated cytokines and chemokines, alterations in autoantibody avidity and G0 carbohydrate content.
- Subjects who are at increased risk for developing future classified RA based on the presence of RA-related autoantibodies demonstrate several other autoimmune and inflammatory characteristics that are similar in nature to individuals with both existing RA and to those known by retrospective analyses to have been in the Preclinical RA period.
- At-risk individuals are characterized by the increased prevalence of asymptomatic small airways disease that may play a key role in initiation of the disease.
- Further studies of at-risk individuals have the potential to deepen the understanding of the initiation and propagation of RA.

Figure 1.

Natural history of RA development. RA progresses through a series of stages first identified and characterized by detectable circulating RA-specific autoimmunity (ACPA+ At-Risk), with or without “arthralgia”, followed by progression through symptoms, signs and clinically apparent and classifiable disease. The presence of bi-directional arrows at early stages indicates that subjects with early disease may resolve the findings and return to an earlier state.

Figure 2.

Stage-specific environmental influences and biomarker alterations found during the evolution of seropositive RA. For earliest factors, current data sets do not allow conclusions regarding potential additional effects on later stages. Support for each is outlined in the text.