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Are enterobacterial common antigens involved in the etiology of rheumatoid arthritis?

S. Aoki

Division of Locomotorial Disorders, Institute for Medical Science of Aging, Aichi Medical University, Aichi-ken, Japan,

Shigehisa Aoki, M.D., formerly Professor and Head, Division of Locomotorial Disorders, Institute for Medical Science of Aging, Aichi Medical University, now Emeritus Professor, Aichi Medical University.

Please address correspondence and reprint requests to: Dr. Shigehisa Aoki, 1206, 5-chome Akaike, Nissin City, Aichi-ken 470-0125, Japan.
E-mail: fwjh6581@mb.infoweb.ne.jp

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovial inflammation and destruction of the articular cartilage and bone (1). Despite many years of intensive research, the initiating factor of rheumatoid inflammation remains unidentified. Many studies have sought to implicate microbes or bacterial components in the etiology of RA showing autoimmune disorder (2). Several investigators (3-5) hypothesize that enteric bacteria are implicated in the pathogenesis of diseases of unknown etiology like RA. Enteric bacteria such as *Clostridium perfringens* (6), *Yersinia enterocolitica* (7), *Proteus mirabilis* (8), and *Escherichia coli* (9) have been implicated, leading to speculations that RA may be a form of reactive arthritis (ReA) (10). However, to date there is no consensus about any of the organisms implicated in the etiology of RA.

Previously, we reported the induction of arthritis resembling RA in rabbits (11) by hyperimmunization with heat-killed *Escherichia coli* O:14 which contained large amounts of enterobacterial common antigens (ECA) (12, 13), and showed that a high proportion of the animals with induced arthritis also had high levels of antibodies to the *E. coli* antigen (14). On the basis of this experiment, more recently we found that patients with RAhave an excess of antibodies against ECAassociated with bacterial outer membrane proteins of 35kDa (OmpA) and 38 kDa (OmpC) commonly found in sera from rabbits with arthritis induced by hyperimmunization with heat-killed *E. coli* O:14, compared with control subjects (15). Furthermore, RA patients with an excess of antibodies to the ECA in synovial fluids (SF) showed typical or characteristic histological features of seropositive RAsynovitis (16).

This paper reviews evidence from the literature and from our own research unit for clinicopathologic, experimental and epidemiological differences and similarities between RA and ReA, and proposes that a subgroup of RA patients with enterobacterial etiology may be present in larger groups of patients with RA, which is thought to be an etiologically heterogeneous disease.

Differences and similarities between rheumatoid arthritis and reactive arthritis

The important hallmarks of RA with a common genetic background, HLA-DR4 (17), are symmetrical chronic polyarthritis, destruction of the joint structures, rheumatoid nodules and rheumatoid factor (RF) in serum. About 80% of patients with RA are RF-positive and this condition is termed seropositive RA, whereas 20% of RA cases are seronegative (seronegative RA) (18). In contrast to seropositive RA, seronegative spondylarthropathies (SpA) associated with HLA-B27 are characterized by involvement of the sacroiliac joints, peripheral inflammatory arthropathy, insertional tendinitis (enthesopathy), absence of rheumatoid nodules and absence of RF, and they include ankylosing spondylitis (AS) as a prototype, Reiter's syndrome (RS), psoriatic arthropathy, juvenile arthropathy, undifferentiated spondylarthropathy and ReA(19).

The term "reactive arthritis" was first used by Ahvonen *et al*. in 1969 (20) to describe the form of aseptic arthritis following gut infection with *Yersinia enterocolitica*. Therefore, ReA is defined classically as a sterile arthritis associated with a localized infection elsewhere in the body (21). ReA is usually caused by enterobacterial infection with *Yersinia*, *Salmonella* and *Shigella*, or genito-urinary infection with *Chlamydia* (21). Up to 80% of individuals with ReA are HLA-B27 positive and therefore at least 20% do not carry this antigen (22). Recently, Toivanen *et al*. (23) proposed two forms of ReA; one HLA-B27-associated form and another HLA-B27-nonassociated form. Moreover, they reported that HLA-B27-associated ReA might appear identical to RS with accompanying urethritis and conjunctivitis and only HLA-B27-associated ReAwas considered to belong to the group of seronegative SpA, whereas in the HLA-B27-non-associated form this had not been clearly described.

ReAis an acute mono- or oligoarticular arthritis in which the joints of the lower extremities are afflicted asymmetrically, in contrast to RA in which symmetric polyarthritis involving the small and

large joints in the upper and lower limbs is seen (24). It is interesting that destruction of the articular cartilage and bone which is characteristic of RA, does not occur in ReA (24). Cooper *et al*. who statistically re-examined sections of synovium from 393 operations on diseased joints, including 9 cases of arthritis in RS and 127 cases of RA by the classic histologic method, found several conspicuous differences between RS and RA (25). For example, the appearance of synovial giant cells, fresh fibrin on the synovial surface and cartilage-bone fragments with an inflammatory reaction in RA were observed in 18.1%, 32.5%, and 9.5%, respectively, whereas all of these lesions were absent in RS (0%). Synovial giant cells, surface fibrin deposits and cartilage-bone fragments suggesting the joint destruction are noted as characteristic histopathologic changes in RA (24, 26, 27).

Contrary to the fact that inflammation of synovium is central to the disease process of RA, inflammation at the site of ligament and capsule insertion (enthesitis or enthesopsthy) is the focus for disease activity in ReAand RS (24). When the literature on clinicopathology in RA and ReA identical to RS was reviewed, it became apparent that RA was different from ReA in several points. However, these differences are considered to be part of the disparity noted between seropositive RA and ReAbelonging to seronegative SpA.

On the other hand, there are patients who are thought to have RA, but are RF-negative. Unlike seropositive RA, patients with seronegative RA tended toward osteosclerosis, destruction without classical erosions, asymmetry and new bone formation (18). This description is similar to the characteristic findings in the SpA summarized by Marzo-Ortega *et al*. (28) Namely, the existence of an overlap between seronegative RA and SpA is suggested. Therefore, the possibility remains that seronegative RA is still being diagnosed as atypical SpA, including HLA-B27-nonassociated ReA.

In regard to the definition and clinical classification of ReA, Toivanen *et al*. (29) stated that the detection of microbial components, including microbial DNA and RNA in the joints of patients with ReA, had lead to worldwide reconsideration. Pacheco-Tena *et al*. (30) reviewed 175 articles containing 110 studies on ReA and 94 studies on RS, and they found that the nomenclature was variable, often confusing and that the diagnostic criteria of one disease tended to overlap with those of the other. Pacheco-Tena *et al.* propose a classification consisting of 3 categories of ReA: probable ReA; definite ReA triggered by bacteria; and bacterial-associated undifferentiated oligoarthritis or SpA. The third category includes a heterogeneous group of patients in whom no clear-cut definition is available. Accordingly, there is a great deal of difficulty in classifying ReA including the undifferentiated group. Hence, it is suggested that ReAitself is an etiologically heterogeneous disease.

Regarding the pathogenetic mechanism of the HLA-B27-associated ReA, Toivanen *et al*. (23) pointed out the similarity between ReA and the experimental antigen-induced arthritis (AIA). AIA is produced by intra-articular injection of antigens in previously immunized animals and is one of the animal models of RA in view of the similarities noted between AIA and RA (31). Accordingly, similarities also exist between RA and ReA as to the animal model.

Concerning laboratory tests for preceding infection at the Third International Workshop on ReA, detection of enteric bacteria such as *Yersinia* and *Salmonel - la* antibodies by enzyme linked immunosorbent assay (ELISA) was listed (32). Gripenberg (7) in 1981 reported that antibodies against *Yersinia entro - colitica* lipopolisaccharide (LPS) could be detected not only in patients with Yersinia arthritis, but also in patients with RA using the ELISA technique and indicated the possibility that some forms of RA might be etiologically linked to *Y. enterocolitica*. In 1985, Ebringer *et al.* reported increased levels of antibodies to *Proteus mirabilis* in the sera of patients with RA from London, but not those with AS or control subjects (8). Subsequently, they published a report in 2003 that antibodies to the *Proteus* microbe had been found in 14 different countries, involving 1,375 RApatients and that the microbe had been isolated from urine cultures of such patients by careful research (33). Such detectable levels of antibodies to the *Proteus* microbe are similar to epidemiological findings indicating the worldwide distribution of RA (34) and ReA (22). Ebringer *et al*. proposed the hypothesis that RA is a form of ReA involving a urinary pathogen, based on the observation that there is some immunological, and in the case of *P. mirabilis*, microbiological evidence linking a member of the Enterobacteriaceae to RA (10). From the facts that RA patients also showed positive antibacterial antibodies similar to ReA patients, an overlap between RA and ReAis suggested etiologically.

Etiology of rheumatoid arthritis with antibodies to enterobacterial common antigens

The present author and co-workers reported in 1996 that patients with RA from Chubu (middle) district in Japan showed significantly increased titers of antibodies against heat-killed *E. coli* O:14 which expressed large amounts of ECA in 39.8% of 83 serum samples and 65.5% of 58 SF samples, compared with controls (healthy donors and osteoarthritis patients) on ELISA (15). Independently, Okamoto *et al*. in 1998 confirmed the presence of antibodies against heat-killed *E. coli* O:14 in 48.8% of 88 serum samples of RA patients from Yokohama (eastern district) in Japan (35). Moreover, RA patients from Osaka (western district) in Japan in 1999 showed almost the same positivity to anti-ECA antibodies as residents of the Chubu district (35). Interestingly, Yokoyama *et al*. (35) reported that patients with systemic lupus erythematodes did not have significantly increased titers of antibodies to heat-killed *E. coli* O:14 compared with control subjects, despite independent confirmation of significantly high titers of the antibodies to heat-killed *E. coli* O:14 in patients with RA from Tokyo. In our further study, immunoblot analysis of the samples from RApatients revealed not only a ladder-like banding

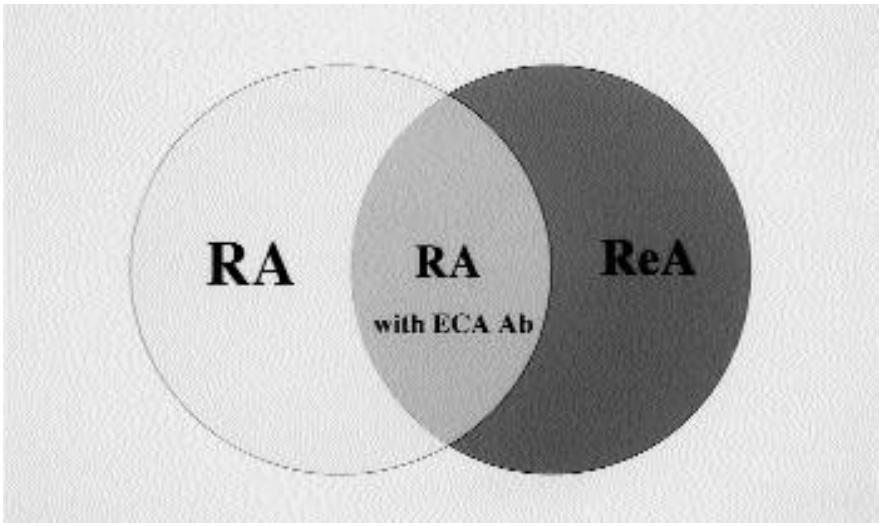

Fig. 1. Diagram showing the overlap between rheumatoid arthritis (RA) and reactive arthritis (ReA). RApatients having anti-ECAantibodies (RAwith ECAAb) that are present within the overlapping area belong to the RAside.

pattern equivalent to ECA associated with LPS (12,13), but also two clear bands of bacterial outer membrane proteins of 35 kDa (OmpA) and 38 kDa (OmpC), having amino acid sequence homology with those of other Enterobacteriaceae (15). These results suggest that some patients with RA are sensitized to antigens common to Enterobacteriaceae (ECA). The ECAof *E. coli* O:14, first described as a cross reacting species by Kunin *et al.* (12), is common to most members of the Enterobacteriaceae family. The author and co-workers first demonstrated the presence of ECA in the cell walls of other *E. coli*, *Krebsiella*, and *Proteus* microbes by an indirect immunofluorescent method using *E. coli* O:14 antiserum (36). Therefore, it is considered that anti-*E. coli* O:14 serum are antibodies against common antigens derived from many sorts of enteric bacteria such as *Krebsiella* and *Proteus* microbes (anti-ECAantibodies).

Therapeutic trials for RA patients having increased titers of anti-ECA antibodies showed a reduction in the antibody levels. As it has been shown that minocycline, a semi-synthetic tetracycline was effective for patients with mild to moderate active RA (37), a clinical research group collaborating with us also carried out administration of minocycline in RA patients with high titers of anti-ECA antibodies and it was found that both anti-ECA antibody levels and RF titers were significantly reduced after administration of minocycline 200 mg/day for 24 weeks (35). The reduction in the antibody levels in RA patients treated with the antibacterial drug suggests the possibility that the disease is associated with continuing enterobacterial infection which triggers RA. On the other hand, fasting has been shown to lead to significant improvement in RA (38). The author and co-workers (35) performed a preliminary study on the effects of a 2 month fast in 15 patients with RA. After treatment with fasting, Lansbury's index as well as anti-ECA antibodies dropped in 3 of 5 patients with RAwho were positive to anti-ECA antibodies and evaluated for Lansbury's index. The results of this study show the correlation between a decrease in ECA antibody levels and a decrease in disease activity, and support an etiologic role for enteric bacteria in RA. Furthermore, it is considered that anti-ECA antibodies may become a marker of joint inflammation in RA patients with anti-ECA antibodies and there is doubt that the antibodies are pathogenic in this disease.

A review of the literature and personal studies have revealed clinicopathologic, experimental and epidemiological evidence to support a hypothesis concerning the existence of an etiologically overlapping area between RA and ReA as shown in Fig.1, despite classifying them as separate entities with definite clinicopathologic differences between the two diseases. Ebringer *et al.* appears to claim that RA with antibodies to *Proteus mirabilis* is thought to exist within the overlapping area in Fig. 1 and that it belongs to the ReA side because they considered that RAis a form of ReA, triggered by *P. mirabilis* (10). In contrast, the present author considers that RA with antibodies to ECA is thought to exist within the overlapping area in Fig.1, belonging to RA side. There are three reasons for this consideration. Firstly, all patients with RAtested for anti-ECAantibodies fulfilled the 1987 revised criteria of the American College of Rheumatology for the Classification of RA (15). Secondly, RA patients having anti-ECA antibodies in SF showed typical or characteristic features of RA synovitis histologically (16). Thirdly, the current author and co-workers previously observed a chronic polyarthritis in rabbits that was induced by hyperimmunization with *E coli* O:14 containing ECA. This animal model closely resembled human RA, not only pathohistologically, but also serologically and immunohistologically (11, 14, 39). Therefore, this result is considered to be experimental evidence for an enterobacterial etiology in RA.

Finally, from the considerations described above, it is suggested that a subgroup of RA patients with an enterobacterial etiology may be present in larger groups of patients with RA, which is thought to be an etiologically heterogeneous disease.

Conclusion

Based on evidence reported previously and also data obtained from personal studies, it is concluded that there is an etiologically overlapping area between RA and ReA, despite classifying them as separate entities with definite clinicopathologic differences between the two diseases. It is considered that RA with antibodies to ECA within the overlapping area between RAand ReA does not belong to ReAwhere autoantibody responses are absent, but to rheu-

matoid disease characterized by autoantibodies. Therefore, the author proposes that a subgroup of RA patients having an enterobacterial etiology may be present in larger groups of patients with RA, which is thought to be an etiologically heterogeneous disease.

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