

Rheumatoid arthritis is linked to *Proteus*—the evidence

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Abstract Rheumatoid arthritis (RA) is a chronic inflammatory arthritic and potentially disabling condition, mainly affecting women of middle age and having characteristic clinical features. Various microbial agents were implicated in the causation of RA. Extensive literature based on the results of various genetic, microbiological, molecular, and immunological studies carried out by independent research groups supports the role of *Proteus mirabilis* bacteria in the etiopathogenesis of RA. New diagnostic markers and criteria and the use of a novel therapeutic protocol in the form of antibiotic and dietary measures are suggested to be used together with current treatments in the management of RA. Prospective longitudinal studies with the use of antimicrobial measures in patients with RA are required to establish the therapeutic benefit of this microbe–disease association.

Keywords *Proteus* · Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory polyarthritic disease associated with remissions and exac-

erations and characteristic genetic, clinical, pathological, and immunological features. The typical clinical presentation of RA is a symmetrical peripheral joint arthritis of insidious onset with positive rheumatoid factors (RF) and progressive erosions of the affected joints occurring predominantly in women of middle age groups. It is a relatively common rheumatic disease with a worldwide distribution. RA causes significant clinical problems and is also responsible for substantial economic and social costs, particularly from work-related disability [1]. In the years between 1979 and 1998, it was observed that more than 20% of deaths from various rheumatic conditions occurred in patients with RA [2].

There are as yet no biochemical or immunological tests with combined high sensitivity and specificity to diagnose and/or monitor the disease activity in RA. This would appear to limit the management of RA patients during the early stages of the disease, especially before the occurrence of irreversible arthritic sequelae. In this review paper we have examined the evidence linking *Proteus* microbes to RA and tried to exploit this association to develop novel diagnostic criteria, which could be helpful in the therapeutic management of patients, especially during the early stages of this disease.

Etiopathogenesis

There is a general consensus that RA results from the interplay of genetic and environmental factors.

Genetic factors

During the last three decades many studies were carried out on the role of genetic factors in the pathogenesis of RA [3].

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RA-associated HLA markers

For decades, a significant genetic contribution for the development of RA was widely accepted and is estimated to account for nearly 60% of the disease risk [4]. Despite extensive studies, no genes other than HLA-DRB1 have yet been more clearly demonstrated to be involved in RA. The association of RA with HLA-Dw4 was first reported in 1976 [5]. With the use of more sensitive techniques, it was later discovered that various HLA-DR4 and non-DR4 alleles are associated with RA among different populations [3]. Of these alleles, HLA-DR4 subtypes (DRB1*0404 and *0405), HLA-DR1 (DRB1*0101), and HLA-DR6 (DRB1*1402) are associated, while other HLA-DR4 subtypes (DRB1*0402 and 0403) would appear not to be linked to RA [6]. This discrepancy in the association of different HLA-DRB1 genes with RA was found to be due to the presence of a conserved hexameric amino acid sequence in the third hypervariable regions of all RA-associated HLA-DRB1 alleles involving positions 69–74 and consisting of glutamic acid, glutamine, lysine or arginine, arginine, alanine, and alanine “EQK/RRAA.” This common sequence in RA was given the name of the “shared epitope” (SE) [7]. More than 90% of RA patients were reported to possess one or more of these alleles, which carry the “SE” moiety [8]. Any theory about the origin of RA must explain this highly significant HLA–disease association. The SE contribution to the development of RA is proportional to the degree of heterogeneity in RA-associated HLA-DR alleles, where it was shown that the relative risk for the development of RA is considerably increased in those with higher numbers of SE-positive sequences [9] present within different RA-associated HLA-DRB1 molecules. In a meta-analytic study of 3,240 patients with RA, an increased association of the SE with the development of erosive disease was observed among many ethnic groups [10].

However, the concordance rate among monozygotic twins with RA is quite low (<15%) [11, 12], suggesting the role of other nongenetic, environmental factors in this condition.

Environmental factors

Among various environmental factors, which were implicated in the causation of RA, infectious agents are the most likely culprits. A variety of viral and bacterial candidates were proposed, but most of these were later discarded because of insufficient evidence.

1. Viruses: Viral infection due to Epstein–Barr virus (EBV), human parvovirus B19, or rubella viruses, each of which was suggested to have a role in RA

etiopathogenesis, usually results in acute polyarthritis of sporadic and self-limiting nature. Furthermore, these viruses are ubiquitous and affect most, if not all, people at some stages in their life.

- 1.1 EBV: EBV is one of the most likely candidates because of the existing evidence for molecular mimicry and positive immune responses associated with these viral agents in patients with RA [13]. However, there are certain problems with this hypothesis. First, it is known that EBV establishes persistent infection in more than 90% of the human adult population worldwide early in childhood [14]. Therefore, it is not unexpected to find enhanced humoral and cellular immune responses in the majority of patients with RA. Second, EBV is the cause of various nonrheumatic diseases, such as infectious mononucleosis and malignancies like Burkitt’s lymphoma and nasopharyngeal carcinoma, and no clear-cut and constant relationship has yet been shown between these conditions and RA [15]. Third, in a prospective study no significant association could be found between positive polymerase chain reaction (PCR) for EBV or CD8 T cell response to EBV and RA disease activity [16]. Fourth, the onset of RA starts predominantly in middle-aged females, while EBV exposure usually occurs in young children before the age of 5 years. Finally, others could not find increased EBV particles in the joint tissues of RA patient [17, 18]. Therefore, the presence of EBV-positive lymphocytes in the synovial membrane is likely to be an epiphenomenon unrelated to the pathogenesis of RA.
- 1.2 Parvovirus B19: Clinical and immunological human [19] and experimental animal [20] studies have supported the role of parvovirus B19 in the pathogenesis of RA, but this could not be confirmed by others [21]. Apart from certain clinical disorders like erythema infectiosum and hydrops fetalis, parvovirus B19 was also implicated in other rheumatic diseases such as systemic lupus erythematosus and being the triggering agent in the production of various nonorgan specific autoantibodies, such as antinuclear, anti-DNA, anti-SSA/SSB, and antiphospholipid antibodies [22].
- 1.3 Rubella and other viruses: A similar lack of evidence occurs with rubella [23], mumps, and measles viruses [24] in RA. In a recent study, patients with RA and those with psoriatic arthritis and reactive arthritis were found to possess signs of replicative viral infections involving EBV and cytomegaloviruses [25].

2. Bacteria

Amid bacterial candidates, apart from *Proteus* microbes, three other bacterial agents have mainly attracted attention in recent years as possible etiological triggers in RA. These include, mycobacteria [26], mycoplasma [27], and *Escherichia coli* [28], but there are some limitations in the proposed role for these bacteria.

- 2.1 Mycobacteria: One of the mycobacterial species is the cause of tuberculosis (TB), a chronic infectious disease affecting the lungs that usually responds to treatment with antimycobacterial therapy. Although no strong epidemiological link has yet been discovered between RA and TB, a mild increase in the risk of encountering TB in patients with RA was reported more recently, but this increase was found to be even higher among patients receiving biological therapies [29]. The suggested triggering mycobacterial antigens in RA, heat shock proteins 65 kD (hsp65), which are also found in other bacterial species [30], showed significant differences in sequences and epitopes with human hsp [31]. Similar immune responses to hsp65 were also found at other inflammatory sites, such as in pleural effusions, which indicates that enhanced reactivity against mycobacterial antigens seems to be a common feature of chronic inflammation [32]. Furthermore, using a PCR gene amplification method, no significant DNA extracts from five mycobacterial species could be detected in the synovial fluid and tissue samples from RA patients [33].
- 2.2 Mycoplasma: Some groups have reported an increased frequency of systemic mycoplasma infection in RA compared to controls [34]. However, a similar prevalence for infection by mycoplasma was also reported in patients with other chronic inflammatory rheumatic diseases, such as spondyloarthritis [35]. Apart from serological evidence of previous mycoplasma exposure in RA patients and in disease controls, other groups were not able to detect mycoplasma DNA in the synovial samples of these patients [36].
- 2.3 *E. coli* and other microbes: *E. coli* shows molecular similarity with RA-associated HLA antigens [37] and patients with RA were shown to have elevated immune responses to the hsp60 antigens of this microbe [38]. However, no significant associations between humoral immune responses to *E. coli* and RA could be shown in more than six other investigations (Table 1).

In a previous study, no clear or specific relationship was found between DNA extracts from single or multiple bacterial species in the synovium from 237 patients with various arthritides, including RA. Ten percent of patient samples were PCR-positive in panbacterial screening assays. Bacterial species identified belonged to the genera *Neisseria*, *Acinetobacter*, *Moraxella*, *Salmonella* and *Pseudomonas*. Thirty-five percent of PCR-positive patients showed the presence of DNA from more than a single bacterial species in synovium [39]. Hence, it seems that RA is not due to the direct invasion of the joints by microbial agents or particles, but could result from the effects of immune responses to triggering microbes residing at remote sites.

“RA” and *Proteus*

Many studies support the role of *Proteus mirabilis* in the etiopathogenesis of RA, and these involve investigations on the microbiological, molecular, and immunological characteristics of these bacteria in patients with RA carried out by independent groups:

- (1) Sera from rabbits immunized with HLA-DR4 positive lymphocytes were found to bind only to *Proteus* species in comparison to 18 other microbes [40].
- (2) HLA-DR4 allogeneic tissue typing sera were binding more significantly to *P. mirabilis* than to *E. coli* microbes [41].
- (3) Molecular similarity was observed between the “ESRRAL” amino acid sequences present in hemolysins of *P. mirabilis* and the “EQ/KRRRAA” SE motif present in the RA-associated HLA-DR molecules [42].
- (4) Molecular similarity was also found between the “LRREI” amino acid sequences present in *Proteus* urease enzymes and the “IRRET” amino acid motif found in type XI collagen [43].
- (5) Antiserum against a 15-mer synthetic peptide containing the RA susceptibility sequence EQ/KRRRAA was shown to bind more significantly to a 16-mer peptide comprising the ESRRAL amino acid sequence from *P. mirabilis* hemolysin molecules, and anti-ESRRAL serum reacted similarly to the EQ/KRRRAA peptides [44].
- (6) Anti-ESRRAL peptide antibodies were found to bind to mouse fibroblast transfectant cell lines expressing HLA-DRB1*0401, but not to HLA-DRB*0402, which is not expressing the SE sequence [44].
- (7) Using ELISA and immunoblotting methods, analysis of urine from 76 patients with RA and 48 healthy controls showed that only 4% of the control urines but 33% of those from the RA patients were infected. The commonest infecting organism in the RA patients’ urine was *P. mirabilis*, which occurred twice as

Table 1 Statistical values of antibodies against various microbial species in patients with RA

Reference	<i>Proteus</i>	<i>Klebsiella</i>	<i>Esch.</i>	<i>Yersinia</i>	<i>Salm.</i>	<i>Chlam.</i>	<i>Shigella</i>	<i>Pseud.</i>	<i>Serratia</i>	<i>Campyl.</i>	NBF spp.	Viruses
Ebringer et al. 1985 [40] ^a	<0.001	NS										
Khalafpour et al. 1988 [67]	<0.05	NS										
Deighton et al. 1992 [68]	<0.001											NS (4) ^b
Subair et al. 1995 [69]	<0.001		NS								NS (3)	
Fielder et al. 1995 [70]	<0.001		NS		NS							
Tiwana et al. 1996 [71]	<0.001		NS					NS	NS			
Tiwana et al. 1997 [72]	<0.001	NS	NS								NS (10)	
Tani et al. 1997 [73]	<0.001	NS	NS									
Wanchu et al. 1997 [55]	<0.001				NS							
Blankenberg-Sprenkels et al. 1998 [74]	<0.001	NS										
Newkirk et al. 2005 [75]	<0.001		<0.05	NS	NS	NS	NS			NS		NS (1)
Rashid et al. 2004 [76]	<0.001		NS						NS			

The *P* values compare patients to controls.

^aReference number

^bNumber of microbes tested

NS Not significant, *EschEscherichia*, *Salm. Salmonella*, *Chlam. Chlamydia*, *Pseud. Pseudomonas*, *Campyl. Campylobacter*, NBF normal bowel flora

frequently as *E. coli* microbes. *P. mirabilis* was found in 52% of the infected urines of the RA patients. Comparison of antibody levels to *P. mirabilis* showed that RA patients had significantly increased levels of IgA, IgG, and IgM antibodies in their sera and urine compared with the control group [45].

- (8) IgG antibodies from RA patients were found to have cytotoxic activities against HLA-DR4-peptide-bearing cells as shown by increased hemolysis for sheep red blood cells coated with HLA-DRB1*0404 peptides comprising the “EQRRAA” amino acid sequences when compared to patients with ankylosing spondylitis and healthy control subjects [46].
- (9) During the last three decades many serological studies were carried out by different independent groups on microbial antibodies against various microbial antigens in patients with RA and other rheumatic diseases. Antibodies against *P. mirabilis* were found to be significantly elevated among a total of 1,375 RA patients, but not in other diseases or healthy subject controls from more than 15 different countries [47].

The high titers of anti-*Proteus* antibodies in RA patients would appear to be specific because there was no such elevation in antibodies against 27 other microbial agents (Table 1).

Upper urinary tract as the main source of *Proteus* infection in RA

A number of studies support a link between upper urinary tract infection (UTI) due to *Proteus* microbes and RA.

- (1) Microbiological studies carried out on 89 RA patients showed that *P. mirabilis* microbes were isolated from the urine of 63% female and 50% male patients, compared to a frequency of isolation in 32% and 11% of healthy women and men, respectively [48]. Furthermore, anti-*Proteus* antibody levels were found to be increased in the urine of RA patients [45].
- (2) Serum anti-*Proteus* antibody levels were found to correlate significantly with the isolation rate of *P.*

mirabilis from the midstream urine specimens of RA patients [49].

- (3) *P. mirabilis* microbes especially those of proticine type 3 strains have a higher affinity to affect the upper urinary tract or kidneys [50].
- (4) *P. mirabilis* is considered to be the second most common causative microbe of UTIs after *E. coli* [51].
- (5) There are reports of increased incidence of UTIs in patients with RA [52, 53]. However, these observations were not confirmed by other groups [54]. This discrepancy in the epidemiological link between urinary infections and RA could be due to the occurrence of subclinical or occult infections, which are merely characterized by bacteriuria. This was recognized in the results of two independent studies in which RA patients were found to have asymptomatic *Proteus* bacteriuria more frequently than healthy controls [45, 48].

It is relevant to note that RA occurs three to four times more often in women than men and women are ten times more likely to have UTIs than men, hence, these observations could explain why RA is more prevalent in women than men.

Molecular mimicry as a possible pathogenetic mechanism in RA

Rheumatic fever is the prototypic disease for molecular mimicry, which is suggested to be one of the main mechanisms involved in the pathogenesis of other immune-mediated rheumatic diseases.

Molecular similarities or cross reactivities exist between *P. mirabilis* and various self-antigens (Fig. 1). Antibodies against these bacterial antigens bind to the tissues carrying these self-antigens in vitro [46].

It should be noted that only a small percentage of HLA-DR4/1 positive individuals are prone to develop RA. It is only individuals with high titers of antibodies against *Proteus* microbes are liable to undergo joint inflammation with consequent pathological damages and development of RA. Patients with inactive (having normal ESR and/or CRP levels) RA, however, do not have elevated anti-*Proteus* antibodies [55].

A positive correlation exists between the levels of antibodies against *P. mirabilis* and the concentration of acute phase proteins in patients with RA [56]. Activation of complements and other inflammatory cascades can only be accomplished in those patients with high anti-bacterial antibody titers because two Fc molecules of IgG are required to activate the C1q component of the complement system.

<i>PROTEUS MIRABILIS</i>	
Haemolysin	Urease
Glu-Ser-Arg-Arg-Ala-Leu	Leu-Arg-Arg-Glu-Iso
Glu-Gln-Arg-Arg-Ala-Ala	Iso-Arg-Arg-Glu-Thr
HLA-DR1/4	Collagen type XI
SELF-ANTIGENS	

Fig. 1 Molecular similarities between *Proteus* and self-antigens

Failure to detect microbial DNA products at the main pathological sites, articular tissues, when analyzed by gas chromatography–mass spectrometry and panbacterial PCR [57] supports the suggestion that the immune system in patients with RA could be triggered by microbes residing at remote sites.

Repeated infections with *Proteus* microbes, which are most likely to be subclinical will result in the production of high titers of bacterial antibodies. The binding of these antibodies to the cross-reactive self-antigens will then trigger a cascade of inflammatory responses sequentially evoking production of various cytokines, chemokines, complement, and proteinases leading to a local inflammation with or without systemic effects. In addition, such activation may have a “bystander” effect on other immune cells, including T lymphocytes, which might result in further enhancement of the inflammatory immune responses, with the development of arthritis and extraarticular and systemic features of this disease.

Proteus infection can explain some prominent features in RA

The involvement of *Proteus*, but not other microbes in RA, could explain some of the characteristic features of this disease.

RA-specific features

- (1) Women in their 30s to 50s are mainly affected by RA and UTIs are more common in females in these age groups.

- (2) RA is most commonly associated with HLA-DR alleles, which carry the SE moieties, and these antigens are cross-reactive with *Proteus* hemolysin.
- (3) RF forms part of the American Council of Rheumatology (ACR) diagnostic criteria for RA. Extensive evidence supports the notion that RFs are produced as the result of triggering microbial infections [58, 59] and *Proteus* microbe could be one of them.
- (4) Characteristic small joints involvement occurs in RA. Elevated levels of antibodies to LRREI peptides present in the *Proteus* urease enzymes, which are cross reactive with IRRET amino acids present in collagen type XI, a component of hyaline cartilage, could explain the small joints involvement.

New diagnostic criteria and therapeutic protocol

Diagnosis

Currently, the diagnosis of either RA is based on the presence of certain clinical and radiological features, where patients can be categorized into different groups according to the ACR criteria [60]. The requirement for using sensitive and specific laboratory diagnostic markers are of crucial importance to identify patients and/or monitor disease activity status during treatment. Various laboratory markers, including RFs, were proposed to fulfill these requirements in RA [61]. These proposed diagnostic markers should be able to identify patients during the early stages of the disease, before the appearances of irreversible pathological sequelae.

We propose that individuals presenting with inflammatory mono/polyarthritis of more than 3 months could be identified as patients with “early RA” if they possess positive SE genetic markers and high anti-*Proteus* hemolysin and urease peptide antibodies [62] (Table 2). These diagnostic criteria could be supported further with an increased isolation of *Proteus* from urinary tract in RA patients. The appearance of other clinical and radiological features of these diseases would be of further help in reaching a final diagnosis.

Treatment

Drugs used in RA are of various types from nonsteroidal anti-inflammatory drugs and disease modifying antirheumatic drugs [63] to biological agents such as antitumor necrosis factor antagonists [64]. However, most, if not all, of these drugs are considered as nonspecific in preventing or stopping the pathological processes in RA. Some of these, especially the biological agents, are expensive [65]

Table 2 Proposed diagnostic criteria for identification of patients with early RA

Criteria for identification of early RA

Early or pre-RA is said to be present in an individual, who has:

- 1) Inflammatory mono/polyarthritis of more than 3-month duration.
- 2) A positive SE amino acid motif.
- 3) Elevated levels of anti-*Proteus* antibodies in the serum, above 95% confidence limit of controls.
- 4) A significant isolation rate of the *Proteus* microbes from midstream urine samples.
- 5) Laboratory signs of inflammation, such as high C-reactive protein and/or increased erythrocyte sedimentation rate.
- 6) No radiological evidence of arthritis.

Advanced or classical RA is said to be present in an individual, who has all features of pre-RA (conditions 1–5) and the radiological evidence of arthritis.

and might possess deleterious or even lethal side effects [66]. Management of RA could be improved through the use of more specific methods aimed at the eradication of *Proteus* microbes, and this could be achieved through the use of antimicrobial therapies involving antibiotics and/or dietary manipulation.

In conclusion, extensive scientific evidence suggests an important role of *Proteus* in RA. The presence of HLA antigens and elevated *Proteus* antibodies in RA could be helpful in the identification of patients with these conditions during early stages of disease. The use of new therapeutic modalities in the form of antimicrobial therapies in conjunction with the currently available treatments should be evaluated in longitudinal studies.

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