Rheumatoid Arthritis as an Autoimmune Disease Caused by *Proteus* Urinary Tract Infections: A Proposal for a Therapeutic Protocol

Taha Rashid MBChB[1], Harmale Tiwana PhD[1], Clyde Wilson PhD[1] and Alan Ebringer MD[1,2]

[1]Division of Life Sciences, Infection and Immunity Group, Waterloo Campus, King's College London and [2]Department of Rheumatology, Middlesex Hospital, UCL School of Medicine, London, UK

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Rheumatoid arthritis is a chronic inflammatory systemic disorder involving peripheral polyarthritis, with or without extra-articular manifestations. The disease has a progressive course characterized by episodes of remissions and exacerbations. RA has been identified in all populations examined, and prevalence figures range between 0.2% and 5.3% [1]. The female predominance compared to males in RA, ratio 4:1, has been given various explanations in terms of hormonal influences and genotypes [2]. However, the most likely explanation for this difference in the sex ratio could be based on variations in anatomical features of the genitourinary tract and, consequently, on the higher frequency of urinary tract infections in women.

Genetic and environmental factors in RA

In the mid 1970s, Stastny [3] reported an association between RA and certain HLA-D region alleles, HLA-DR4-Dw4. More recently, it has become apparent that certain subtypes of HLA-DR4 predominate in patients with RA. HLA-DR4 can be divided into at least five subtypes – Dw4, Dw10, Dw13, Dw14, Dw15 – which differ in sequence by only a few amino acids, primarily between positions 69 and 74, located along the alpha helical rim of the peptide-binding cleft. This amino acid motif consists of glutamic acid, glutamine, arginine, arginine, alanine and alanine or "EQRRAA" residues. This sequence is found in HLA-DR1 and HLA-DR4 subtypes – Dw14 and Dw15 – while in DR4-Dw4 subtype, lysine replaces the arginine at position 71 to become the "EQKRAA" amino acid sequence, but the overall stereochemical structure closely resembles the "EQRRAA" sequence [4]. By contrast, other DR4 subtypes – such as DR4-Dw10 which has an aspartic acid and glutamic acid replacement at positions 70 and 71 respectively, and DR4-Dw13 which has a glutamic acid residue at position 74 – change the molecular and charge characteristics of this region; interestingly, these HLA-DR4 subtypes do not confer increased susceptibility to RA.

Patients with RA who are HLA-DR4 negative but carry other HLA-DR molecules, such as DR1, DR6, or DR10, were also found to possess the "EQK/RRAA" amino acid sequence except for DR10, which has an arginine residue at position 70 that does not seem to alter the overall structural moiety of this epitope. This observation led to the proposal of the "shared epitope hypothesis" to explain the association of different HLA antigens with RA [5]. The distribution of these RA-associated genetic markers differs among various populations. HLA-DRβ1*0401 (Dw4) and DRβ1*0404 (Dw14) are more common in Caucasians, DRβ1*0405 (Dw15) in Japanese, DRβ1*0101 (DR1-Dw1) in Israelis, DRβ1*1402 (DR6-Dw16) in American Yakima Indians, and DRβ1*1001 (DR10) in Spanish populations [review in 6].

Taking all these "EQK/RRAA" positive RA-associated HLA molecules into consideration, it was observed that up to 96% of RA patients exhibit at least one of these alleles [7], providing further support to the SE hypothesis. This structural moiety is therefore suggested to be somehow directly involved in the disease pathogenesis. Based on this common feature, RA patients can be separated into those who are positive (SE+ve) and negative (SE-ve) for this amino acid motif regardless of the type of the HLA molecule that they may possess. Furthermore, the identification of the SE sequence alone by using commonly applied methods such as polymerase chain reaction and oligonucleotide sequencing could be sufficient to recognize individuals with an increased risk of developing this disease. The SE sequence identification could be used only in those patients presenting with inflammatory arthritis in association

RA = rheumatoid arthritis

SE = "shared epitope"

with certain diagnostic criteria for whom early treatment with more specific therapeutic measures may be required.

In spite of this strong genetic link, the low concordance rate of 15% in RA monozygotic twins [8] in combination with a low disease penetrance among different families of patients with RA strongly support the role of non-genetic environmental factors, particularly microorganisms in the etiopathogenesis of this disease.

Various microbial species have been suggested to be involved in the etiology of RA. At present the most likely microbial candidate that could trigger disease development would appear to include *Proteus mirabilis* [9] and *Escherichia coli* [10] enterobacteriae. However, in several studies carried out by our and other independent groups, no increase in antibody titers to *E. coli* was found, but in each case a significant elevation of antibodies to *P. mirabilis* was detected in RA patients [11–14].

Molecular mimicry and the role of *Proteus* in the etiopathogenesis of RA

Nearly three decades ago the findings of studies carried out by two independent groups, one from Israel [15] and the other from the USA [16], suggested the possibility of the existence of a link between *Proteus* bacteria and RA, and over the last 16 years compelling evidence has accumulated to support this association. The results of these studies are: firstly, antibodies against *P. mirabilis* were found to be significantly elevated in RA patients when compared to controls. Independent studies from more than 10 countries [review in 17], and most recently from Russia [18], have confirmed this association. Secondly, there is evidence of cross-reactivity between *P. mirabilis* and autoantigens based on the results of both immunological and molecular studies, which can be summarized as follows:

- A rabbit immunized with HLA-DR4-positive lymphocytes and tested by immunodiffusion methods against soluble extracts obtained from 18 different microbes showed binding with *Proteus* species to a greater extent than with other bacterial agents [9].
- Allogeneic human tissue-typing sera tested against *P. mirabilis* and *E. coli* using the enzyme-linked immunosorbent assay method were found to bind *Proteus* to a significantly greater extent than non-DR4 tissue-typing sera, while no such increased binding was observed with *E. coli* microorganisms [19].
- A sequence similarity between RA-associated HLA molecules possessing the SE motif (positions 69-74) and the amino acid sequence "ESRRAL" (positions 32-37) found in the membrane hemolysins of *Proteus* bacteria was identified and observed to have a similar stereochemical shape as the SE sequence [20].
- Antibody levels against a 16-mer synthetic peptide containing the SE sequence were found to be significantly elevated in Japanese RA patients when compared to healthy controls [21].
- Antibodies against a 16-mer synthetic peptide containing the "ESRRAL" amino acid motif from *Proteus* hemolysins but not to three other control peptides were found to be significantly elevated in British RA patients when compared to healthy controls [22].
- A structural homology was found to be present between the *Proteus* urease enzyme molecule containing an "IRRET" amino acid sequence (positions 337-341) and the amino acid sequence "LRREI" (positions 421-425) present in the collagen type XI [Table 1], a component of hyaline cartilage that is commonly found in the small joints of the hands and feet. RA patients appeared to have elevated antibody titers to preparations of both *Proteus* hemolysins and urease enzymes [22]. Therefore, a double pathological effect would appear to be present: firstly, anti-*Proteus* hemolysin antibodies bind to the SE sequence of synovial cells in susceptible individuals, and secondly, anti-*Proteus* urease antibodies would appear to bind to type XI collagen present in hyaline cartilages especially around the edges of the synovial capsule from where blood vessels emerge and rheumatoid erosions are encountered.
- Antibody analysis against random peptide phage libraries has shown a significant elevation of antibodies to "ESRRAL" sequence-containing peptides among Norwegian RA patients when compared to healthy controls [23]. Although various microorganisms possess "SE-like" sequences, significantly elevated levels of antibodies were found only against *Proteus* but not against the other microorganisms in patients with RA [14].

The most plausible mechanism for the development of this disease could be the molecular mimicry hypothesis, which has also been suggested to explain the role of *Klebsiella pneumonia* in the etiopathogenesis of ankylosing spondylitis [24]. This model suggests that antibodies that result from *Proteus* infection could bind, although with lower affinity, to the cross-reacting self-

Table 1. Cross-reactive antigens present in *P. mirabilis* strain

Proteus antigens/ Peptides (positions)	Self-antigens/ Peptides (positions)	Distribution and sources of self-antigens
Hemolysins/ESRAAL (32-37)	HLA-DR4-Dw4 DRβ1*0401/EQKRAA (69-74) HLA-DR4-Dw14, Dw15, and HLA-DR6-Dw16 DRβ1*0404,*0405, and DRβ1*1402/EQRRAA (69-74) HLA-DR10 DRβ1*1001/ERRRAA (69-74)	All nucleated cells, especially B cells and other activated immune cells including fibroblasts, chondrocytes and synoviocytes
Urease/IRRET (337-341)	Collagen type XI/LRREI (421-425)	Hyaline cartilage mainly in the peripheral joints

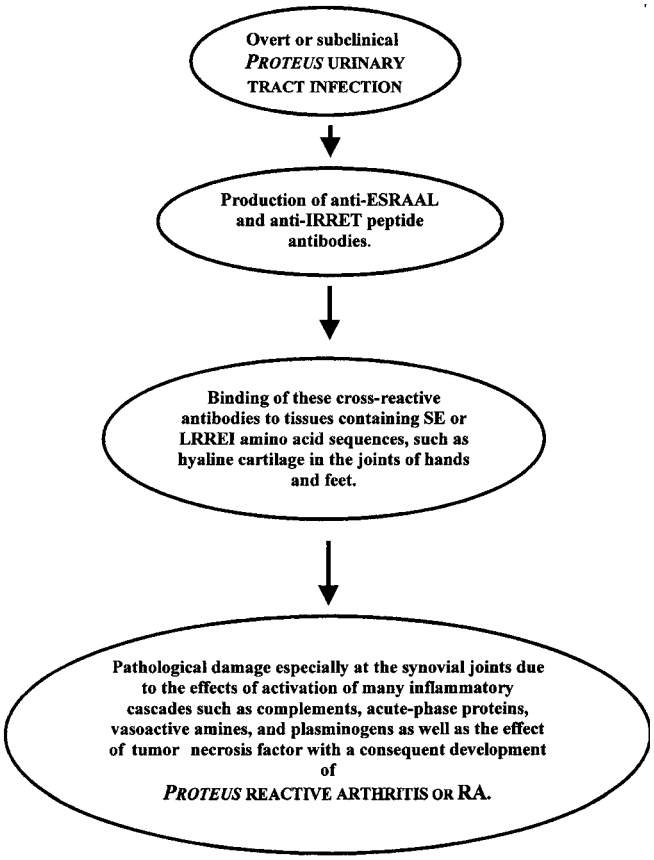

Figure 1. The sequential pathological process in the development of RA following *Proteus* infection

antigens residing in the areas of the pathological lesions, particularly the joints [25]. The cytotoxic effects of these antibodies could be produced by activating inflammatory cascades such as acute-phase proteins, components of the complement family and vasoactive amines, or the action of natural killer cells, as well as various cellular cytotoxic products, such as cytokines and degradative enzymes [Figure 1].

Proteus as a source of overt or subclinical urinary tract infection in RA

The increased isolation rate of *P. mirabilis* from the urine of RA patients was first reported in 1993 by our group at the "Sixth International Seminar on the Treatment of the Rheumatic Diseases" held in Tel Aviv, Israel [26] [Figure 2]. A significant correlation was found between urinary isolation of *Proteus* bacteria and the anti-*Proteus* antibody levels in RA patients [27]. Also reported was an increased incidence of urinary tract infections in patients with RA and Sjögren's syndrome [28], which could possibly indicate a role of *Proteus* in this disease and at the same time explain the higher incidence of RA in women compared with men. It seems that a proportion of RA patients may show signs of overt urinary tract infection or at least have asymptomatic bacteriuria, suggesting a clinical or subclinical exposure to *Proteus* bacteria.

Proteus species, particularly the *P. mirabilis* strain, is known to be the second most common cause of urinary tract infection after *E. coli*, with a predilection for the upper urinary tract, especially the medulla of the kidneys [29]. Owing to their ability to produce urease enzyme, these microorganisms will raise the urinary pH with a consequent formation of struvite and apatite microcrystals, leading to the formation of renal stones [Figure 3]. These bacteria have the ability to persist in the upper urinary tract and are difficult to eradicate, especially when a bacterial nidus is established in a calculus. These bacterial sources could act as a prolonged antigenic stimulus, giving rise to persistently elevated antibody titers especially during the active phases of RA.

A new proposed management protocol

Currently there are two major categories of drugs used in the management of patients with RA.

The first group consists of non-steroidal anti-inflammatory drugs, which modulate or depress some manifestations of inflammation. These therapeutic drugs are all considered as non-curative in that they do not reverse or halt the disease process but only alleviate the symptoms.

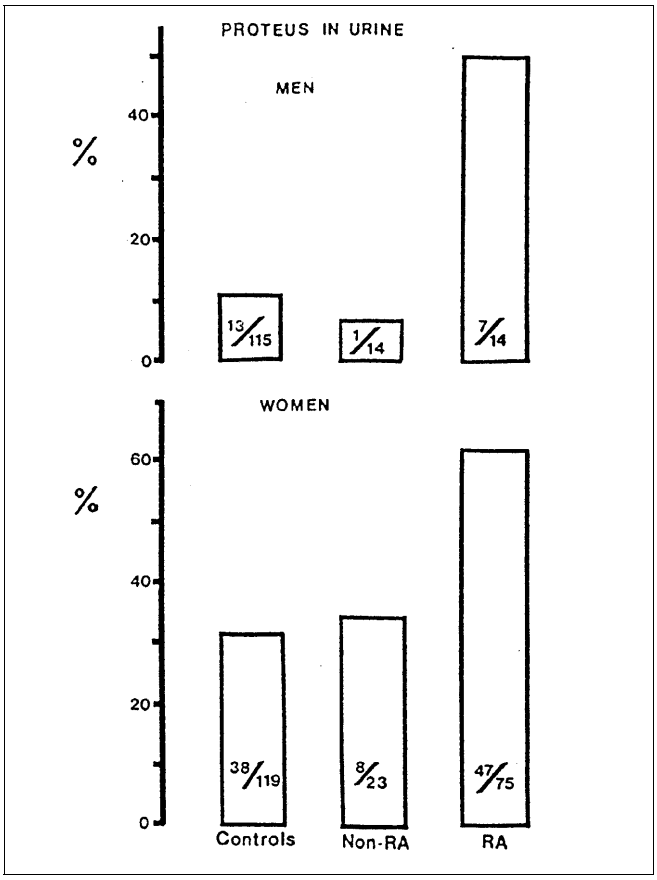

Figure 2. Isolation rate (%) of *Proteus* from the urine of RA and non-RA patients and healthy controls [adapted from Ebringer et al., 26]

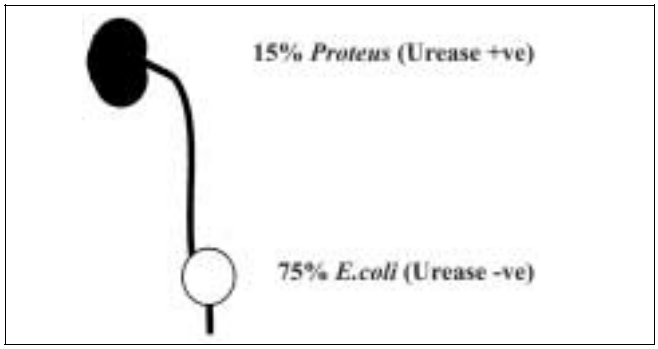

Figure 3. Frequency of upper and lower urinary tract infection and urease production by *P. mirabilis* and *E. coli* microorganisms

The second group of agents, which are the disease-modifying anti-rheumatic drugs, appear to act on a more fundamental level in the mechanism of inflammation. These second-line treatment drugs include antimalarials, D-penicillamine and gold salts, which are used alone or in combination with steroids and some immunosuppressive or cytotoxic preparations, such as methotrexate.

Both types, however, have serious side effects and may produce adverse reactions. It is suggested, therefore, that the implementation of other more specific anti-*Proteus* therapies with or without the use of the NSAIDs or DMARDs could have a beneficial and synergistic, if not a curative, effect in this disease. The following specific antimicrobial measures are being proposed as novel modalities of therapy in the treatment of RA [Table 2]:

Antibiotics

Antibiotics have been tried many times in the management of RA. Among these, sulphasalazine, metronidazole, rifampicin as well as minocycline [30], have all been shown to produce some beneficial results. It would appear reasonable to use a method whereby the causative agent, *Proteus* bacteria, could be eradicated by using antibiotics to which this microbe is sensitive. *In vitro* and *in vivo* studies have shown significant responses of *Proteus* microbes to various antibiotics such as ciprofloxacin, norfloxacin or trimethoprim with or without sulfamethoxazole (TMP-SMX) [31], or more effectively in combination trials of amoxycillin and ampicillin or amoxycillin and aminoglycosides such as gentamycin, tobramycin and netilmicin [32]. The use of such antibiotics in RA requires clinical evaluation in well-controlled prospective studies.

Diet therapy

Dietary measures in the form of a vegetarian diet with or without fasting was shown to have some specific effects in reducing the levels of antibodies to *Proteus* but not to *E. coli* in RA patients [33]. This diet could be supplemented with cranberry juice [34], which was shown to have specific antibacterial effects by preventing these microbes from binding to the upper urinary tract epithelium and thereby avoiding the persistence of infection. Zafriri and co-workers from Tel Aviv [35] confirmed these findings and also found that fructose, which is present in the cranberry juice cocktail, is one of the essential compounds that inhibit bacterial adhesions to the uro-epithelium. High intake of cranberry juice results in increased concentrations in the urine of hippuric acid, which is known to have antibacterial activity. In a clinical controlled trial among a group of elderly people, daily consumption of a cranberry juice cocktail (1% fructose) in comparison with a placebo beverage (no fructose) for 6 months significantly decreased the prevalence of bacteriuria. The same study also noted trends toward decreased antibiotic use and fewer symptomatic urinary tract infection episodes in the treatment group [36].

Other therapeutic measures

- High fluid intake (2 L daily) with voiding at 2 or 3 hourly intervals by day may have a flushing effect on the kidneys. Cox and Hinman [37] demonstrated that good hydration and urine flow is important for maintaining sterilization in the urinary tract.
- Acidification of the urine, by intake of vitamin C (ascorbic acid) or hexamine (methenamine), and restriction of alkaliz-

Table 2. Therapeutic principles in RA

Treatment lines	Time of commencement	Treatment period
Antibiotics		
Ciprofloxacin or Norfloxacin or TMP-SMX or Amoxicillin+Ampicillin or Amoxicillin+Aminoglycosides, such as gentamycin, tobramycin, etc.	Early cases with positive treatment criteria	2–4 weeks (according to severity). Repeated with any sign of overt or subclinical *Proteus* urinary tract infection.
Diet		
Cranberry juice (CJ) Cranberry juice cocktail (=CJ+glucose+fructose) Vegetarian diet	With or after antibiotic therapy	Throughout the disease period
High fluid intake (2–4 L/day)		
Mainly water and soft drinks	With or after antibiotic therapy	Throughout the disease period
Acidification of urine		
Vitamin C Hexamine and/or Decreased intake of milk and other alkalizing products	With or after antibiotic therapy	Throughout the disease period

NSAIDs = non-steroidal anti-inflammatory drugs
DMARDs = disease-modifying anti-rheumatic drugs

ing agents such as milk or sodium bicarbonate may help to lower the urinary pH [38]. Chronic infection by *Proteus* species, which are known to possess urea-splitting properties with production of ammonia, will result in the elevation of urine pH, leading to the formation of urinary stones through precipitation of struvite and apatite crystals [39]. Acidification of the urine may therefore help in reducing the size and number of the infected urinary calculi and hence in decreasing the frequency of recurrent urinary tract infections caused by these microorganisms.

- Surgical removal of the infected urinary stones is of great importance in the management, since, if present, these renal calculi may act as a source of persistent urinary tract infections and hence interfere with the effects of the chemotherapeutic agents.

Requirements for early diagnosis and management in RA patients

The best time to start with the proposed antibacterial therapeutic measures is during the early stages of the disease, and that means before the main inflammatory processes have resulted in permanent irreversible arthritic damage. The most suitable individuals to receive such measures are those who carry the disease-susceptibility genetic markers.

Early diagnosis is important. Firstly, the use of ciprofloxacin in RA patients during the late stages of the disease was shown to have no beneficial effects [40]; and secondly, in early cases of arthritis when the classical features of RA diagnosis are not met, the differential diagnosis may include other diseases like reactive arthritis, gout, systemic lupus erythematosis or many other related disorders.

Identification of susceptible cases and the early use of specific therapeutic measures in the pre-destructive stages of the disease is therefore of crucial importance. The identification of such cases may be established by the presence of the SE sequence and two of the following three diagnostic laboratory criteria:

- Presence of high titer of anti-*Proteus* antibodies
- Isolation of *Proteus* bacteria from the urine of these patients
- Presence of inflammatory disease activity markers such as high C-reactive proteins or elevated erythrocyte sedimentation rate.

Conclusion

The time has come for introducing more specific curative measures in patients with RA. This can be achieved with a comprehensive, double-blind prospective study in a group of SE+ve patients presenting with inflammatory mono/polyarthropathy and who also possess the necessary criteria required for the application of this management protocol.

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Correspondence: Dr. A. Ebringer, Division of Life Sciences, Infection and Immunity Group, King's College London, Stamford St., London SE1, UK. Phone: (44-20) 7848-4302, Fax: (44-20) 7848-4500,
email: alan.ebringer@kcl.ac.uk