

Worldwide Links between *Proteus mirabilis* and Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is a systemic and arthritic autoimmune disease affecting millions of people throughout the world. During the last 4 decades extensive data indicate that subclinical urinary tract infection by *Proteus mirabilis* has a role in the aetiopathogenesis of RA based on cross-reactivity or molecular mimicry between Proteus haemolysin and RA-associated HLA-DRB1 alleles as well as between Proteus urease and type XI collagen. Studies from 15 countries have shown that antibodies against Proteus microbes were elevated significantly in patients with active RA in comparison to healthy and non-RA disease controls. Proteus microbes could also be isolated more frequently in the urine of patients with RA than in controls. It is suggested that treatment of RA by using antibiotics and increased daily fluid intake in order to eradicate Proteus bacteria from urinary tract could be implemented in conjunction with the currently used immunosuppressant's and biologicals.

Keywords: Rheumatoid arthritis; *Proteus mirabilis*

Introduction

Rheumatoid arthritis (RA) is a common arthritic disorder affecting millions of people throughout the world with women 2-3 times more likely to be affected than men. The prevalence of RA was estimated in the region of 1.3 to 2.1 million [1]. RA could have a considerable impact on the social, psychological [2] and economic [3] conditions of the patients. RA could be associated with extra-articular manifestations and other co-morbidities which might have a negative impact on the patient's quality of life [4]. A general scientific consensus suggests that RA is an immune-mediated disease that could be triggered by an environmental (mainly microbial) factor in a genetically susceptible individual.

Genetic Involvements in RA

In an important report Peter Stastny from Dallas, showed that some 70% of RA patients belonged to the HLA-DR4 group whilst its frequency in the general population was only about 30% [5]. This result was confirmed by many other centres throughout the world [6]. However, the roles of other, but less predominant genes have not been ruled out [7].

The concordance rate in monozygotic twins was estimated to be 15% among English [8], 12.3% Finnish [9] and 9.1% Danish [10] populations, thereby suggesting the involvement of environmental factors.

The mechanism behind the development of RA could be due to molecular mimicry between an external agent and the HLA-DR4 group, in the same way as there is an association between streptococcal tonsillitis and rheumatic fever-associated conditions such as Sydenham's chorea, arthritis and carditis [11].

Investigation into the Link between HLA-DR4 and Bacteria

Serum was obtained from a rabbit prior to immunisation to be used as a control sample. The rabbit was then immunised with lymphocytes obtained from a patient with severe RA, whose HLA-DR tissue type was DR4,X. Serum samples were tested against soluble extracts from 18 different bacteria (Table 1).

| Microorganism | Culture Reference Number |
|--------------------------------|--------------------------|
| Gram-Negative Bacteria | |
| <i>Proteus vulgaris</i> | QEC-B11 |
| <i>Pseudomonas aeruginosa</i> | QEC-B15 |
| <i>Proteus mirabilis</i> | QEC-B17 |
| <i>Alcaligenes faecalis</i> | QEC-B18 |
| <i>Shigella sonnei</i> | QEC-B21 |
| <i>Salmonella typhimurium</i> | QEC-B22 |
| <i>Enterobacter cloacae</i> | QEC-B27 |
| <i>Escherichia coli</i> | QEC-B35 |
| Gram-Positive Bacteria | |
| <i>Staphylococcus albus</i> | QEC-C1 |
| <i>Staphylococcus aureus</i> | QEC-C22 |
| <i>Streptococcus faecalis</i> | QEC-D2 |
| <i>Streptococcus lactis</i> | QEC-D8 |
| <i>Streptococcus pyogenes</i> | QEC-D10 |
| <i>Streptococcus viridians</i> | QEC-D15 |

| | |
|--------------------------------|--------|
| <i>Bacillus subtilis</i> | QEC-S6 |
| <i>Staphylococcus aureus</i> | STHC-1 |
| <i>Streptococcus pyogenes</i> | STHC-2 |
| <i>Streptococcus pneumonia</i> | STHC-3 |
| Yeast | |
| <i>Candida albicans</i> | STHC-4 |

Table 1: Microorganisms used in the immunodiffusion studies. (QEC = Queen Elizabeth College collection, STHC = St. Stephen’s Hospital collection)

Rabbit antiserum gave 5 precipitin lines against *Enterobacter cloacae*, *Salmonella typhimurium*, *Alcaligenes faecalis*, as well as against *Proteus mirabilis* and *Proteus vulgaris* [12]. However, the pre-immunisation serum also produced precipitin lines against the first three microbial sonicates, namely *Enterobacter cloacae*, *Salmonella typhimurium* and *Alcaligenes faecalis*.

The “HLA-DR4 immunised” rabbit serum produced precipitin lines against *Proteus* bacteria, microorganisms which are the second commonest cause of urinary tract infections after *Escherichia coli*, especially in the upper urinary tract. It became apparent that a urinary tract infection could readily explain the preponderance of RA in women.

Proteus bacteria are ubiquitous in nature, they are found in soil, on vegetables, in water, sewage, mammalian gut and vagina [13]. The suggestion arises that *Proteus* bacteria may be involved in RA and if this hypothesis is correct then antibodies to this microbe should be demonstrable in RA patients.

Locations of RA patients showing High *Proteus* Antibody Levels

Various independent groups from 15 different locations worldwide have been able to show that antibodies against *Proteus mirabilis* antigens were higher among RA patients when compared to the corresponding patients with other diseases or healthy controls from the same populations (Table 2).

England

Serum samples were obtained from 30 RA patients attending the weekly “Gold Clinic” at the Middlesex Hospital in London. Their mean age was 59 years (Range: 40-78 years) and the diagnosis was made according to the American Rheumatism Association criteria [14]. Antibody titres to *Proteus mirabilis* were measured by a Coombs agglutination assay [12]. The mean *Proteus mirabilis* agglutination titre in the RA patients was found to be significantly higher than the level found in controls ($p < 0.001$).

A group of 31 English RA patients attending the Lister Hospital in Stevenage were also studied, and antibodies were measured. The RA patients with active disease were also found to have significantly elevated levels of IgG *Proteus* antibodies when compared to the level in English control subjects ($p < 0.001$), but there was no elevation of antibodies to *Escherichia coli* [15].

Ireland

Active RA patients attending the Rheumatology Clinic of St. Vincents Hospital in Dublin, Ireland were investigated for the presence of antibodies to *Proteus mirabilis* and compared to patients with coeliac disease, systemic lupus erythematosus, or sarcoidosis, as well as healthy controls. Antibody levels were measured by ELISA. The levels of antibodies to *Proteus* in 29 RA patients were significantly elevated ($p < 0.001$) when compared to healthy blood donors as well as when compared to the 3 disease groups: coeliac disease, sarcoidosis, and systemic lupus erythematosus patients [16].

| No. | Country (Location) | RA patients | Controls* | P values** | Reference No. |
|-----|-----------------------------------|-------------|-----------|--------------|---------------|
| 1 | England | 30 | 41 | $p < 0.001$ | 12 |
| | (Winchester) | | | | |
| | (London) | 27 | 25 | $p < 0.001$ | 20 |
| | (Epsom) | 60 | 60 | $p < 0.001$ | 45 |
| | (Newcastle) | 142 | 121 | $p < 0.0001$ | 46 |
| | (Stevenage) | 66 | 60 | $p < 0.001$ | 47 |
| 2 | Ireland (Dublin) | 28 | 16 | $P < 0.01$ | 16 |
| 3 | Scotland (Dundee) | 100 | 100 | $P < 0.0001$ | 17 |
| 4 | France | 50 | 49 | $P < 0.001$ | 18 |
| | (Brest) | | | | |
| | (Toulouse) | 15 | 49 | $P < 0.001$ | 18 |
| 5 | Netherlands (Amsterdam) | 25 | 34 | $P < 0.001$ | 19 |
| 6 | Norway (Oslo) | 53 | 30 | $P < 0.001$ | 20 |
| 7 | Spain (Barcelona) | 34 | 14 | $P < 0.001$ | 20 |
| 8 | Finland (Helsinki) | 72 | 30 | $P < 0.001$ | 21 |
| 9 | Japan | 30 | 23 | $P < 0.05$ | 21 |
| | (Tokyo) | | | | |
| | (Otsu) | 50 | 50 | $P < 0.001$ | 22 |
| 10 | Russia (Moscow) | 27 | 100 | $P < 0.0001$ | 23 |
| 11 | India (Chandigarh) | 70 | 82 | $P < 0.001$ | 25 |
| 12 | Taiwan (Taichung) | 39 | 51 | $P < 0.001$ | 26 |
| 13 | Bermuda (Hamilton) | 34 | 33 | $P < 0.001$ | 15 |
| 14 | USA (Philadelphia & Bethesda) and | 113 | 133 | $P < 0.0005$ | 27 |
| 15 | Canada (Montreal) | | | | |
| | Total numbers | 1065 | 1101 | | |

Table 2: Geographical locations where patients with rheumatoid arthritis (RA) have shown significant elevations in antibodies against *Proteus* or cross-reactive antigens when compared to the corresponding controls of healthy subjects or non-RA patients *Controls are either healthy individuals or non-RA patients. **P values

are referred to statistical results of the levels of antibodies against Proteus or cross-reactive antigens in RA patients compared to controls

Scotland

Serum samples tested for anti-bacterial antibodies, which have been collected from 100 rheumatoid factor-positive patients with various autoimmune diseases and joint pains, were compared to sera from 100 rheumatoid factor-negative patients attending the Ninewells Hospital in Dundee. The results were shown that RF-positive sera had significantly higher levels of IgM antibodies to *P. mirabilis* when compared to those of the RF-negative sera ($p < 0.0001$). This response was subsequently found to be associated with sera from patients who clinically had RA. Furthermore, RA patients sera showed to have a significantly higher ($p < 0.02$) IgM antibody levels to *P. mirabilis* than to other organisms tested, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [17]. The IgM response to RA patients was not specific for only one O serotype of *P. mirabilis* but was associated with all eleven O serotypes tested. This antibody response was also found to be independent from C-reactive protein and RF.

France

Sera from 50 tissue-typed, active RA patients and 49 healthy controls were obtained from Brest in France. These sera were tested for antibodies against 3 different bacteria, *Proteus mirabilis*, *Escherichia coli* and *Salmonella typhimurium* [18]. The active RA patients from Brest were observed to have elevated levels of IgG Proteus antibodies when compared to the level in control subjects ($p < 0.001$). There was no elevation in IgG antibody titres against *Escherichia coli* or *Salmonella typhimurium*. Sera were also collected from 15 patients with active RA in Toulouse. Serum anti-Proteus antibodies in 15 RA patients from Toulouse were measured by indirect immunofluorescence. The mean (\pm standard error) anti-Proteus antibody titre in the RA patients from Toulouse was 6.30 ± 0.34 and this difference was again statistically significant when compared to the blood donors from Brest ($p < 0.001$) [18].

Netherlands

Antibody studies were carried on Dutch RA and ankylosing spondylitis patients with and without acute anterior uveitis. There were 25 active RA patients whose mean ESR was 56 mm/hour. They showed significant elevations in IgG antibody titre against Proteus when measured by immunofluorescence, compared to either HLA-B27 positive controls ($p < 0.001$) or to HLA-B27 negative controls ($p < 0.001$) [19]. HLA-B27 positive patients with active ankylosing spondylitis showed the highest levels of IgA antibodies to *Klebsiella pneumoniae* and this was significantly higher than in HLA-B27 positive healthy controls ($p < 0.001$) or HLA-B27 negative healthy controls ($p < 0.001$) or in RA patients ($p < 0.001$). RA patients had antibodies to *Proteus*, whilst ankylosing spondylitis patients had antibodies to *Klebsiella*, thereby each disease group was a specificity control for the other condition.

Norway and Spain

In one study, serum samples from RA patients in three different locations, Barcelona in Spain, Oslo in Norway and Epsom in England were examined to determine the levels of anti-Proteus IgG antibodies compared to the corresponding healthy control subjects using indirect

immunofluorescence technique: 34 Spanish RA patients recruited from Barcelona, 53 Norwegian RA patients from Oslo, while the third group consisted of 27 English RA patients from Epsom. The results showed that active RA patients had a significantly higher level of anti-Proteus antibodies than their corresponding healthy controls among the Spanish ($p < 0.001$), Norwegian ($p < 0.001$) or English populations ($p < 0.001$) [20].

Finland

There were 129 sera obtained from a Finnish population consisting of 72 patients with "Early RA" and 27 patients with "Advanced RA" attending the Second Department of the Medicine of the University of Helsinki. "Early RA" was defined as a disease that was present for less than 12 months and "Advanced RA" was a disease present in a patient for more than 12 months. Antibodies were measured using indirect immunofluorescence against *Proteus mirabilis*, *Serratia marcescens* and *Escherichia coli*. Significantly elevated levels of Proteus IgG antibodies were found in both the "Early RA" ($p < 0.001$) and "Advanced RA" ($p < 0.001$) compared to healthy Finnish controls [21]. Similar elevations were found in the Proteus IgM antibodies in both the "Early RA" ($p < 0.02$) and "Advanced RA" ($p < 0.001$) compared to the corresponding healthy controls, but no such elevations were found in the IgA class. No significant immunoglobulin elevations were found against *Serratia marcescens* or *Escherichia coli*.

Japan

Thirty patients with active RA were recruited from the Otsu region in Japan to be examined for determination of antibodies against different microbes. ELISA studies were carried out against *Proteus mirabilis*, *Escherichia coli* and *Klebsiella pneumoniae*. Patients with active and probably active RA showed elevated levels of IgG antibodies against *Proteus mirabilis* when compared to controls ($p < 0.001$) [22]. Active RA patients also had elevated levels of IgM antibodies against *Proteus mirabilis* when compared to controls ($p < 0.001$). There were no IgG, IgA or IgM antibody elevations in active or probably active RA patients when tested against *Klebsiella pneumoniae* or *Escherichia coli*.

Russia

In a study from Moscow 27 patients with RA was examined for the levels of antibodies against *Proteus mirabilis* and compared to 100 healthy control subjects from the same area. Significant elevations of anti-Proteus IgM and IgG antibodies were observed ($p < 0.0001$) in patients with RA compared to healthy subjects [23]. In another study from the same location antibody levels were examined in the sera of 42 Russian RA patients against *Proteus mirabilis* and two serotypes (O3 and O9) of *Yersinia enterocolitica*. It was found that increased IgG antibody levels were noticed in sera of RA patients when tested against both Yersinia serotypes, especially O3, whilst it was observed that IgM isotypic antibodies against Proteus microbes were more significantly increased among the RA patients group [24]. Increased levels of antibodies against both microbes in patients with RA could be possibly due to the cross-reactive antigens shared between these microbes.

India

Seventy patients with active or inactive RA from Chandigarh in India were studied for determination of the levels of antibodies against *Proteus* and *Salmonella* microbial antigens. Other groups in the study

that have been recruited from the same area included 18 patients with osteoarthritis and 82 healthy control subjects [25].

Antibody titres against *Proteus* antigens were found to be significantly higher in active patients with RA when compared to patients with osteoarthritis or to healthy controls ($p < 0.001$). However there was no significant difference in anti-Salmonella antibody levels among various disease and control groups.

Taiwan

Serum samples from 39 patients with RA compared to 52 patients with ankylosing spondylitis attending the China Medical College Hospital in Taichung and 51 corresponding healthy controls were examined for class-specific antibodies against *Proteus mirabilis*, *Escherichia coli*, *Campylobacter jejuni*, *Klebsiella pneumoniae*, *Yersinia enterocolitica*, *Salmonella typhimurium* and *enteritidis* microbes [26].

Although RA patients showed elevations of IgA isotypic antibodies against all bacterial strains, mainly IgG class antibody levels against *P. mirabilis* ($p < 0.001$) and *K. pneumoniae* ($p < 0.01$) were found to be significantly elevated when compared to healthy subjects.

Bermuda

Sera were obtained from 34 patients with active RA in Bermuda having erythrocyte sedimentation rates of more than 15 mm/hour. Samples were also obtained from 33 healthy controls. Antibodies were measured by ELISA against *Proteus mirabilis* and *Escherichia coli* [15]. The Bermudian RA patients with active disease had an elevated mean level of IgG *Proteus* antibodies when compared to the level in Bermudian control subjects ($p < 0.001$). However, there was no elevation in antibodies to *Escherichia coli*.

USA and Canada

A multicenter cohort study was carried out on 246 patients with early inflammatory arthritis (<1 year) attending health centers at Montreal or Winnipeg in Canada and the VA Medical Centre in Philadelphia. Among these patients 113 were identified to have RA, 75 of who were rheumatoid factor-positives. Other groups included in the study were 43 patients with spondylarthropathy and 90 patients with undifferentiated arthritis [27].

The levels of IgM and IgA antibodies against *Proteus mirabilis* were found to be significantly higher mainly in rheumatoid factor-positive RA patients group when compared to patients with spondylarthropathy ($p < 0.0005$) and undifferentiated arthritis ($p < 0.0005$).

Evidence of *Proteus* Urinary Tract Infections in RA

The microbe *Proteus mirabilis* accounts for only about 10% of all urinary tract infections [28]. However, in some studies the interesting observation was made that no elevation was found in antibodies against the commonest microbe causing about 80% of urinary tract infections namely, *Escherichia coli* [29]. The elevation of antibodies only against *Proteus mirabilis* indicated that such immune responses

were specific and could not be ascribed to non-specific urinary tract infections occurring in patients whose manual dexterity had been compromised by arthritis or age. Thus, a question arises whether patients with RA have evidence of infections with *Proteus mirabilis*?

Increased incidence of urinary tract infections in RA have been reported in two independent studies from Edinburgh [30] and Tel Aviv [31]. These findings suggest that there is a possibility for an existing hidden infection expressed in the form of asymptomatic bacteriuria involving *Proteus* microbes in patients with RA [32].

Therefore, an investigation was carried out to determine whether the urine of patients with active RA contained *Proteus* bacteria. In a controlled study of 89 RA patients from London, *P. mirabilis* was isolated more significantly in females (63%) and males (50%) in comparison to age and sex-matched healthy control females (32%) ($p < 0.001$) and males (11%) ($p < 0.001$). However, no significant increase in the isolation rates of *Proteus* microbes were observed in urine of patients with other rheumatic diseases when compared to healthy control subjects [33].

In another study carried out by a group from Dundee in the U.K., a significantly increased isolation rate of *Proteus* microbes from the urine of 76 patients (33%) were detected when compared to those of 48 gender-matched healthy individuals (4%) [34].

Molecular Mimicry, HLA-DR4 and “Shared Epitope” Hypothesis

The association between RA and some sub-types of HLA-DR4 is well established. In Caucasian subjects DR4/Dw4 and DR4/Dw14 subtypes are associated with RA, whereas in Japanese subjects it is DR4/Dw15 that is the susceptibility factor [35]. In Israel where DR4/Dw10 predominates, an association with HLA-DR1 has been reported in patients with RA [36].

Analysis with synthetic oligonucleotides has shown that a particular region of the DR β 1 chain, from positions 70-74 coding for amino acids Gln-Arg-Arg-Ala-Ala (QRRAA), specific for DR1, Dw14 and Dw15, showed a strong association with RA compared to control subjects [37]. The sequence closely resembles that found in DRB1*0401 (DR4/Dw4) individuals, there being only one conservative substitution at position 71, from arginine to lysine (QKRAA). These two amino acids are positively charged and thus the overall shape and charge configuration of these two sequences are similar. This sequence Q(K)RRAA has been described as the “shared epitope” by the Winchester group from New York [38], and this link between RA and “shared epitope” has been confirmed by various other groups over the last two decades [39]. The glutamic acid (E) occupying position 69 is common to all DR β 1 molecules. Furthermore the EQRRAA sequence is also found in DRB1*1402 (DR6/DW16) positive Yakima Indians affected by RA. The RA susceptibility sequence spanning residues 69-74 (EQRRAA) was used to scan published sequences of molecules from *Proteus* microorganisms. A closely related sequence (ESRRAL) spanning residues 32-37 of the surface membrane haemolysin of *Proteus mirabilis* (Hpm B polypeptide) was identified which had biochemical and charge similarity to the susceptibility sequence (Figure 1).

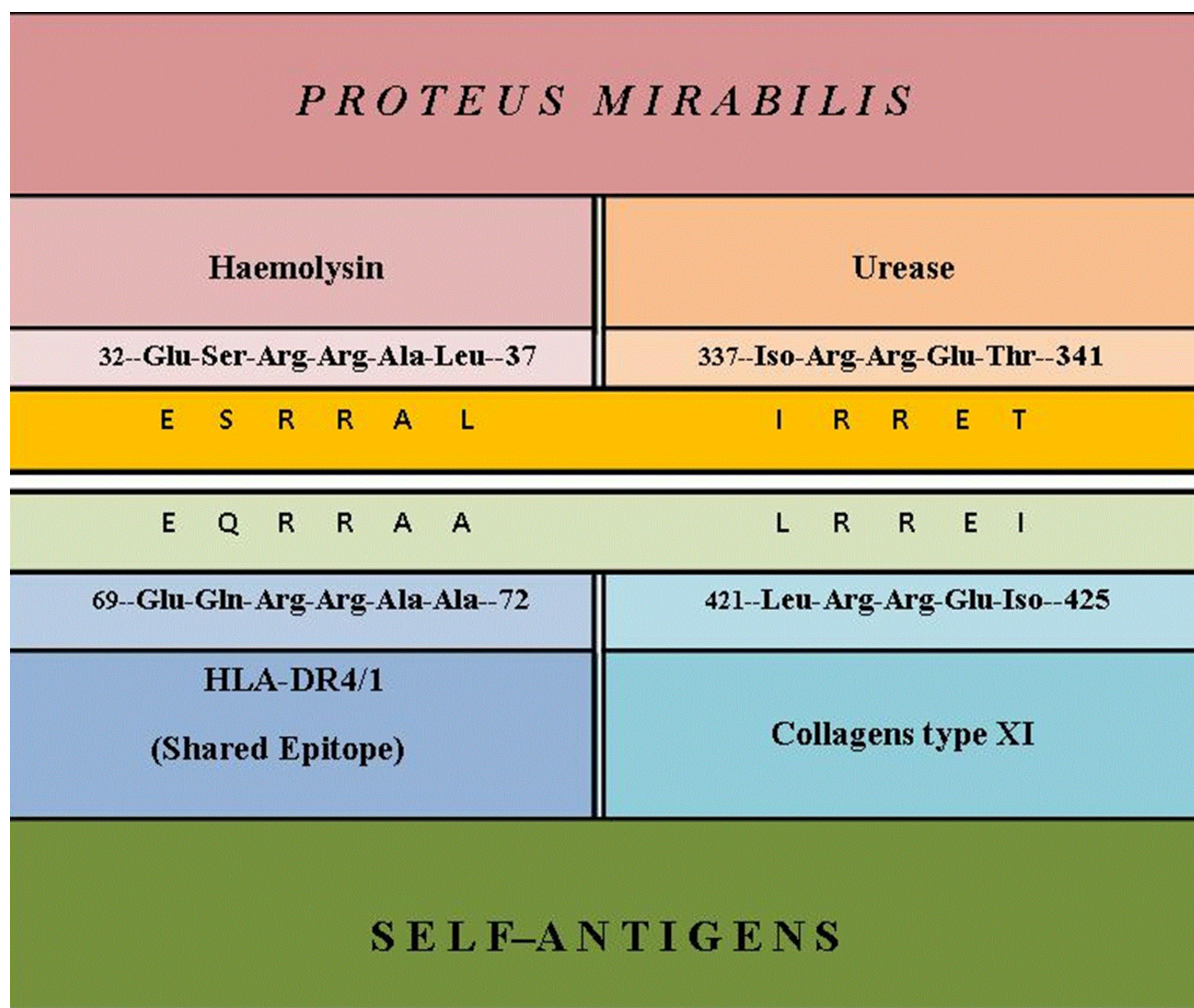


Figure 1: Schematic picture depicting molecular similarities between *Proteus mirabilis* and self-antigens

Molecular Mimicry between Proteus Urease And Hyaline Cartilage

Cartilage destruction is a major feature of the disease. A search of the protein database was made for any sequence of *Proteus mirabilis* showing structural similarity to any collagens. One way of distinguishing urine cultures is by determining whether the bacterial colonies are either urease positive or urease negative. Urease is not present in *Escherichia coli* but is present in Proteus bacteria. Thus the hypothetical question arose if the Proteus urease molecule had any similarity to collagens known to be present in joint tissues.

A computer analysis showed an amino acid homology between Proteus mirabilis urease (IRRET), amino acid residues 337-341 and $\alpha 2(XI)$ collagen (LRREI) residues 421-425 (Figure 1) [40,41]. Type XI collagen is a component of hyaline cartilage which is composed of three different polypeptide subunits $\alpha 1(XI)$, $\alpha 2(XI)$ and $\alpha 3(XI)$. This

suggests that antibodies to these two Proteus cross-reactive self-components may be involved in the onset of RA.

Proteus Antibodies in RA Patients have Cytotoxic Activity

In an endeavour to assess the cytotoxic activity of antibodies sheep red blood cells were coated with 15-16 mer peptides containing either the (EQRRAA) or (LRREI) sequences, which are homologous to antigens found in Proteus haemolysins or ureases respectively [42].

It was observed that the percentage lysis of sera from RA patients against cells coated with (EQRRAA) peptides was significantly higher when compared to sera from patients with ankylosing spondylitis ($p < 0.0001$) or healthy controls ($p < 0.0001$) (Figure 2). Similar results were also found when RA patient's sera have been tested against cells coated with (LRREI) peptides.

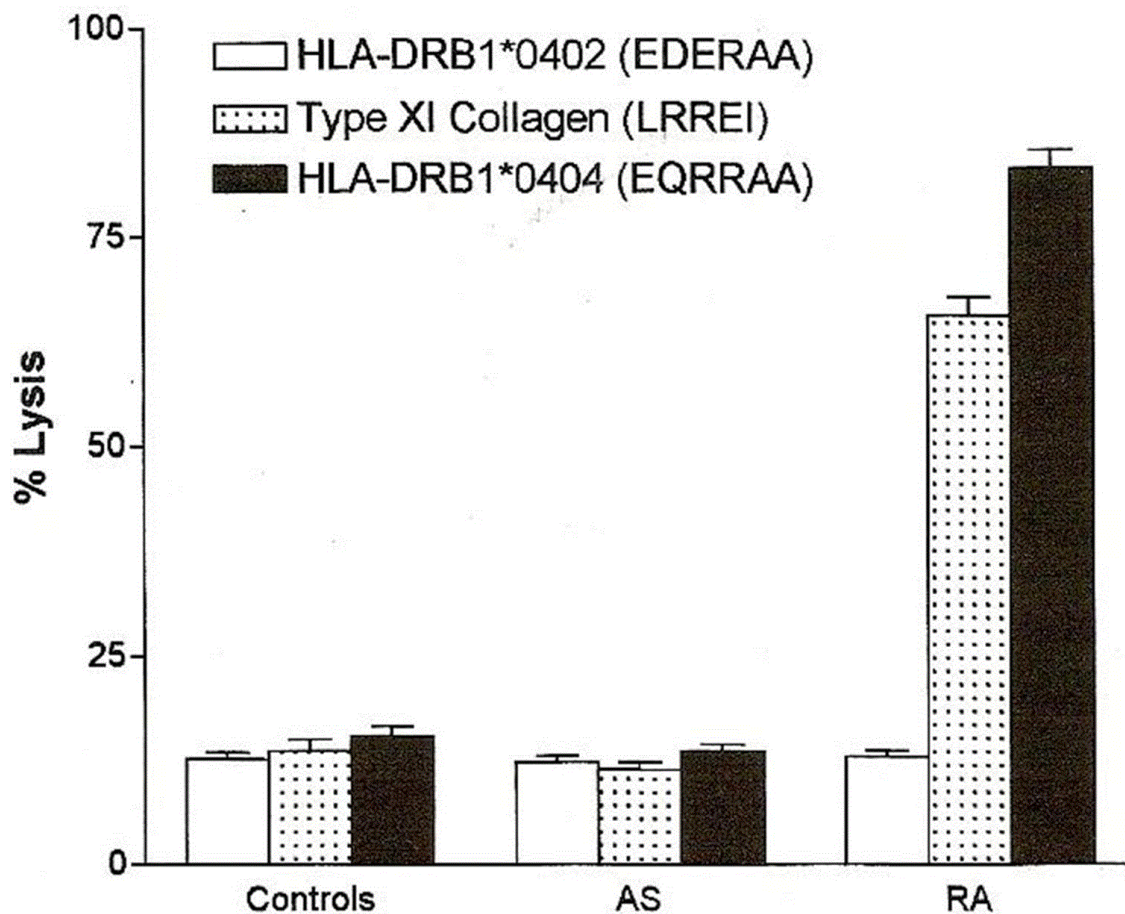


Figure 2: Percentage lysis of sheep red blood cells coated with HLA-DRB1*0404 (EQRRAA), DRB1*0402 (EDERAA – an HLA sequence not linked with RA) and type XI collagen (LRREI) peptides via complement mediated cytotoxicity by control sera, and sera from ankylosing spondylitis (AS) and RA patients. (With kind permission from Wilson et al. 2003)

Conclusion

Antibodies to the urinary microbe *Proteus mirabilis* have been demonstrated to be present in RA patients from several countries. Molecular mimicry between *Proteus* haemolysin and HLA-DR4 and between *Proteus* urease and hyaline cartilage, provide a possible explanation for the pathogenesis of the disease. Moreover, anti-*Proteus* antibodies have cytopathic properties against joint components. Since several studies have shown that antibiotics, such as minocycline [43], sulfasalazine, clarithromycin and levofloxacin [44-47] are effective in the treatment of RA, the question arises whether multi-centre studies should be carried out using *Proteus*-sensitive antibiotics together with a high fluid intake as well as the currently used medical treatments including biologicals, to assess their therapeutic effectiveness in this disease.

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