Chapter 14
Gram-Negative Bacteria Possess Sequences Which Resemble the 'Shared Epitope' but Only *Proteus* Infect Rheumatoid Arthritis Patients

Contents

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Introduction: The ESRRAL Sequence of *Proteus* Haemolysin Is Also Found in Three Other Gram-Negative Bacteria

The 'shared epitope' EQRRAA has been found to resemble or show 'molecular mimicry' with the sequence ESRRAL spanning residues 32–37 of the surface membrane haemolysin of *Proteus mirabilis* (Hpm B polypeptide).

The same hexamer sequence was also found in the membrane haemolysin of *Serratia marcescens* (Shl B polypeptide).

Furthermore, homologous sequences were also found in *Escherichia coli* (QKRAA) and in *Pseudomonas aeruginosa* (DQRRAA).

This study was undertaken to investigate whether active rheumatoid arthritis patients have antibodies to these other Gram-negative microorganisms.

Sera from Rheumatoid Arthritis Patients and Controls

Sera were obtained from active rheumatoid arthritis patients, having an erythrocyte sedimentation rate greater than 15 mm/h and attending the Rheumatology Department at the Lister Hospital in Stevenage. Sera were also obtained from active ankylosing spondylitis patients attending the 'Ankylosing Spondylitis Research Clinic' of the Middlesex Hospital. The diagnosis of rheumatoid arthritis was according to the American Rheumatism Association criteria and that of ankylosing spondylitis by the New York criteria. Sera from healthy control subjects were supplied by the Blood Transfusion Service in London.

In the study, total immunoglobulin antibodies were measured in 181 individuals against *Serratia marcescens*, *Proteus mirabilis*, *Escherichia coli* and *Pseudomonas aeruginosa*.

The groups examined were as follows: 60 patients with rheumatoid arthritis (21 male; 45 female) with a mean age of

48 years (range: 20–71 years) and having a mean (± standard error) erythrocyte sedimentation rate 43.4 ± 4.3 mm/h. The female to male ratio was 2.1:1.

There were also 61 ankylosing spondylitis patients (49 male; 12 female) with a mean age of 46 years (range: 24–73 years) and having a mean (± standard error) erythrocyte sedimentation rate 42.9 ± 9.5 mm/h and 60 healthy control subjects (30 male; 30 female) with a mean age of 34 years (range: 19–66 years).

Enzyme Immunosorbent Assay (ELISA)

Serratia marcescens, *Proteus mirabilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were clinical isolates obtained from the Department of Microbiology at King's College, London. Cultures were prepared and ELISA carried out, as previously described.

The C-reactive protein levels were determined by the single radial immunodiffusion method of Mancini and the results expressed as mg/L of serum.

Antibody Results Against the Four Gram-Negative Bacteria

Antibodies to *Proteus mirabilis* of total (IgA + IgG + IgM) immunoglobulin were significantly elevated in the active rheumatoid arthritis patients compared to active ankylosing spondylitis patients or healthy controls. The mean ± (standard error) in rheumatoid arthritis patients was 0.869 ± 0.054 OD units and this was significantly higher than the mean in active ankylosing spondylitis patients which was 0.228 ± 0.012 ($t = 11.30$, $p < 0.001$) or the mean in healthy control subjects which was 0.214 ± 0.015 ($t = 11.22$, $p < 0.001$) (Fig. 14.1).

There was no significant difference in antibody levels between the active ankylosing spondylitis patients and healthy controls.

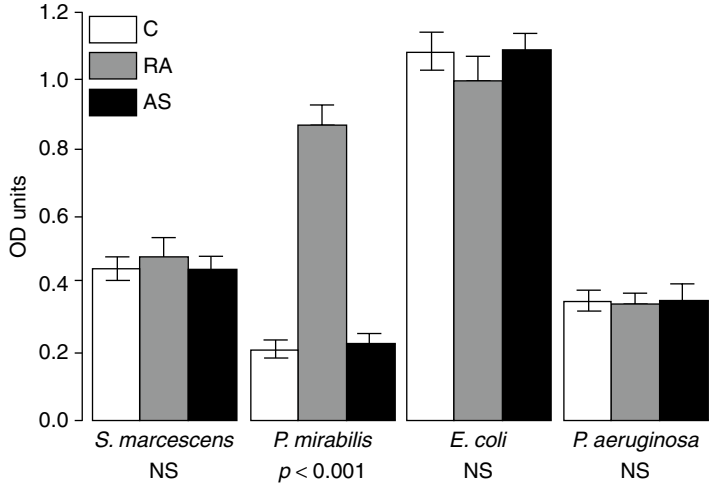

FIGURE 14.1 Total immunoglobulin titres (mean ± standard error) in *Serratia marcescens*, *Proteus mirabilis*, *Escherichia coli* and *Pseudomonas aeruginosa* in 60 healthy controls (*C*), 60 active rheumatoid arthritis (*RA*) and 61 active ankylosing spondylitis (*AS*) patients. (*N.S.* not significant) (Reprinted with kind permission from Tiwana et al. (1996))

There was no significant reactivity in the rheumatoid arthritis or ankylosing spondylitis patients against *Pseudomonas aeruginosa*, *Serratia marcescens* or *Escherichia coli* when compared to the healthy controls (Fig. 14.1).

The reproducibility of the assay for each sample was tested by calculating the coefficient of variation. The coefficients of variation for *Serratia marcescens*, *Proteus mirabilis*, *Escherichia coli* and *Pseudomonas aeruginosa* were calculated to be 4%, 5%, 7% and 4% respectively.

The mean ± (standard error) C-reactive protein level was significantly higher in both the rheumatoid arthritis patients 40.4 ± 9.4 mg/l ($t = 7.29$, $p < 0.001$)(range: 0–160) and ankylosing spondylitis patients 34.0 ± 3.3 mg/l ($t = 5.85$, $p < 0.001$) (range: 0–108) when compared to the mean in controls which was 12.0 ± 1.6 mg/l (range:1–37).

Discussion and Conclusions

In this study, active patients with rheumatoid arthritis have been shown to have an increased levels of total immunoglobulin (IgA + IgG + IgM) against whole *Proteus mirabilis* microorganisms but no elevations against the three other Gram-negative bacteria namely *Serratia marcescens*, *Escherichia coli* and *Pseudomonas aeruginosa* which are known to carry ESRRAL or a homologous sequence.

It has been reported that rheumatoid arthritis patients with early disease exhibit high antibody titres to a 15-mer synthetic peptide of the dnaJ heat shock protein of *Escherichia coli* (Albani et al. 1995) This heat shock protein also contains the EQKRAA sequence found in the susceptibility sequences of HLA molecules associated with rheumatoid arthritis.

The susceptibility sequence EQKRAA is also found in the dnaJ heat shock protein of Epstein–Barr virus and a mimicking sequence DQRRAA is also found in the isoamylase precursor of *Pseudomonas aeruginosa* with a conservative substitution of glutamic acid (E) for aspartic acid (D) (Table 14.1).

However, there does not appear to be any antibody elevations in rheumatoid arthritis patients against either *Escherichia coli* or *Pseudomonas aeruginosa*.

The ESRRAL sequence is also present in *Vibrio cholerae* and *Brucella ovis* (Protein Information data base (PIR) release 44). However, these microorganisms are highly pathogenic and therefore unlikely to persist in patients following recovery. It is unlikely that they play a role in the onset of a chronic disease.

For a chronic autoimmune disease, one requires a mildly pathogenic microbe, causing almost an asymptomatic disease with a continuous and florid production of antibodies which bind to cross-reactive self-antigens and when present in high titres will activate the complement cascade and NK cells which then lead to tissue damage at a site distal from the original site of infection. Such anti-bacterial antibodies binding to self-tissues will then be known as autoantibodies.

Table 14.1 Comparison of amino acid sequences of HLA-DRβ1 chain, spanning residues 69–74 and microorganisms retrieved from PIR release 44 which have similar sequences in other proteins

Source	Amino acids	Positions	Location
DRβ1 chain	EQRRAA	69–74	DR1, DR4/Dw14, Dw15, DR6/Dw16
Epstein–Barr virus	EQRRAA	807–812	dnaJ heat shock protein
E. coli	EQRRAA	61–65	dnaJ heat shock protein
P. aeruginosa	DQRRAA	579–584	dnaJ heat shock protein
P. mirabilis	ESRRAL	32–37	Haemolysin B precursor
S. marcescens	ESRRAL	34–39	Haemolysin (Shl B)
V. cholerae	ESRRAL	19–24	Methyltransferase-adenine specific precursor
B. ovis	ESRRAL	107–112	Isoamylase precursor

PIR Protein Information Resource data base

This model occurs in rheumatic fever, and it is not inconceivable that a similar process might occur with rheumatoid arthritis patients when they produce high titres of anti-*Proteus* antibodies.

Further evidence for a role of microorganisms in the aetiology of rheumatoid arthritis is illustrated by the use of antimicrobial agents such as minocycline which have been shown to reduce joint tenderness and swelling and also a decrease in inflammatory parameters such as C-reactive protein and erythrocyte sedimentation rates (Langewitz et al. 1992).

In a double-blind placebo-controlled trial, a group from the Netherlands have shown that minocycline is effective in some patients with rheumatoid arthritis (Kloppenburg et al. 1994).

Finally one must mention Professor Thomas McPherson Brown (1906–1989) who graduated from the John Hopkins Medical School in Baltimore (Maryland) and spent his lifetime investigating rheumatoid arthritis. Whilst at the Rockefeller Institute in New York, he developed the concept that antibiotic therapy, especially minocycline, might be beneficial in the treatment of rheumatoid arthritis. His results have been confirmed by many centres, the question arises, which bacteria were involved, mycoplasma or the *Proteus* bacteria as suggested by these studies. Both mycoplasmas and *Proteus* bacteria respond to minocycline therapy, and clearly further investigations are required to resolve these questions.

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