Chapter 7
Dutch Patients with Rheumatoid Arthritis Have Antibodies to *Proteus*

Contents

The Netherlands Connection: An Introduction

In the early 1980s, the Immunology Unit of King's College had shown that there were elevated levels of antibodies to the Gram-negative bowel microbe *Klebsiella* in patients suffering from ankylosing spondylitis.

A. Ebringer, *Rheumatoid Arthritis and Proteus*, 63
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In 1992, I was attending a Rheumatology Congress in Barcelona when the late Professor Bert Feltkamp from Amsterdam approached me and said: 'Alan, we have done 3 studies in the Netherlands and we cannot find antibodies in our Dutch ankylosing spondylitis patients as you do in London. It must be the London water'. Clearly, here was a challenge as I knew there was nothing wrong with the London water.

I said: 'Bert, you code the Dutch ankylosing spondylitis and uveitis patients in Amsterdam and send them to us in London. We will send you the results back to Amsterdam and you can decode them'.

'For good measure, send us also sera from active, rheumatoid arthritis patients who have an erythrocyte sedimentation rate greater than 30 mm/h (ESR > 30 mm/h) at the time the blood sample is taken, as we have also found the probable cause of this disease'.

'It is caused by a urinary tract infection by *Proteus* bacteria'. The sera were sent and the studies were carried out, using an immunofluorescence assay. For ankylosing spondylitis, we used an IgA anti-*Klebsiella* assay, whilst for rheumatoid arthritis, we used an IgG anti-*Proteus* assay.

Amsterdam: Location and History

Amsterdam is the capital and largest city of the Netherlands with a population of about two million. The city is in the province of North Holland, but the Ophthalmic Research Institute collects patients from the whole of the Netherlands.

Its name is derived from Amstellerdam, the city on the dam of the river Amstel. It started as a fishing village, but in 1306 was granted city rights. It is not as old as other Dutch cities such as Rotterdam, Utrecht and Nijmegen. However, it flourished in the Middle Ages, because it traded with the Hanseatic League.

In the sixteenth century, the Dutch rebelled against Philip II of Spain which led to the 'Eighty Years War' at the end of which Netherlands gained its independence.

The seventeenth century is considered Amsterdam's 'Golden Age' when it traded all over the world.

The Amsterdam merchants founded the Dutch East India Company and opened the first stock exchange in the world.

During the Second World War, the Dutch people suffered a severe famine. It was noticed by Dutch doctors that patients with coeliac disease improved when there was a shortage of wheat. It led eventually to the discovery that gliadin, a component of wheat, was involved in this disease.

Patients and Controls

The incidence of 'acute anterior uveitis' in HLA-B27-associated ankylosing spondylitis is about 30%. Furthermore, about half of patients with HLA-B27-positive acute anterior uveitis fulfil the criteria of ankylosing spondylitis or reactive arthritis (Linssen et al. 1983). Characteristics of the patients and controls are summarized in the Table 7.1.

The first three groups of patients with acute anterior uveitis had a complete ophthalmological assessment. The patients in the fourth group had ankylosing spondylitis but had never complained of eye problems and therefore were not examined by an ophthalmologist.

The sex ratio was almost equal in the acute anterior uveitis patients who did not have ankylosing spondylitis.

However, in the patients with ankylosing spondylitis, the male/female ratio was about 2/1.

The sex ratio was not determined in the rheumatoid arthritis patients.

The sera from ankylosing spondylitis, acute anterior uveitis and rheumatoid arthritis patients as well as controls were numbered in Amsterdam in such a way that the laboratory in London was not aware of their origin.

The code was broken in Amsterdam after the serological results arrived back from London.

Table 7.1 Characteristics of the six groups of patients and the two groups of healthy controls

	AAU only			**AS**		**RA**	**Controls**	
Number of subjects	17	17	17	17	17	25	17	17
Male/female	7/10	8/9	11/6	15/2	12/5	ND	8/7	5/7
Mean age (years)	46	44	37	38	44	ND	37	32
Mean ESR (mm/h)	ND	ND	ND	ND	ND	56	ND	ND
Mean CRP (mg/l)	4.9	7.3	11.0	12.0	25.3	25.8	5.9	4.2
HLA-B27	+	–	+	+	+	ND	+	–
Acute anterior uveitis (AAU)	+	–	+	–	ND	ND	–	–

Due to identity protection, sex and age were not known for some controls
ESR erythrocyte sedimentation rate, *CRP* C-reactive protein, *AS* ankylosing spondylitis, *ND* not done

Serum C-Reactive Protein Levels

Serum C-reactive protein levels were measured by the single radial immunodiffusion method of Mancini.

Serum C-reactive protein levels in the two control groups were relatively low (Table 7.1).

Serum C-reactive protein levels were found to be highest in HLA-B27-positive patients with active ankylosing spondylitis and patients with active rheumatoid arthritis when compared to all other disease groups or controls.

Of the remainder, only two groups showed any significant elevation in C-reactive protein levels. The HLA-B27-positive, acute anterior uveitis–positive, ankylosing spondylitis–positive patients had higher levels of C-reactive protein when compared to HLA-B27-positive controls ($t = 1.60$, $p < 0.05$). The other group consisted of HLA-B27-positive, acute anterior uveitis–negative, ankylosing spondylitis–positive patients who also had an elevated level of C-reactive protein ($t = 2.14$, $p < 0.025$). Furthermore, HLA-B27-positive, acute anterior uveitis–positive, ankylosing spondylitis patients had a higher level of C-reactive protein when compared to HLA-B27-negative controls ($t = 2.02$, $p < 2.60$). A similar significant elevation in C-reactive protein level was observed in the HLA-B27-positive, acute anterior uveitis–negative, ankylosing spondylitis–positive patients when compared to the HLA-B27-negative controls ($t = 2.60$, $p < 0.005$).

Indirect Immunofluorescence Studies with *Proteus* and *Klebsiella* in Rheumatoid Arthritis, Ankylosing Spondylitis and Acute Anterior Uveitis Patients from the Netherlands

Indirect immunofluorescence assay was carried out as previously described.

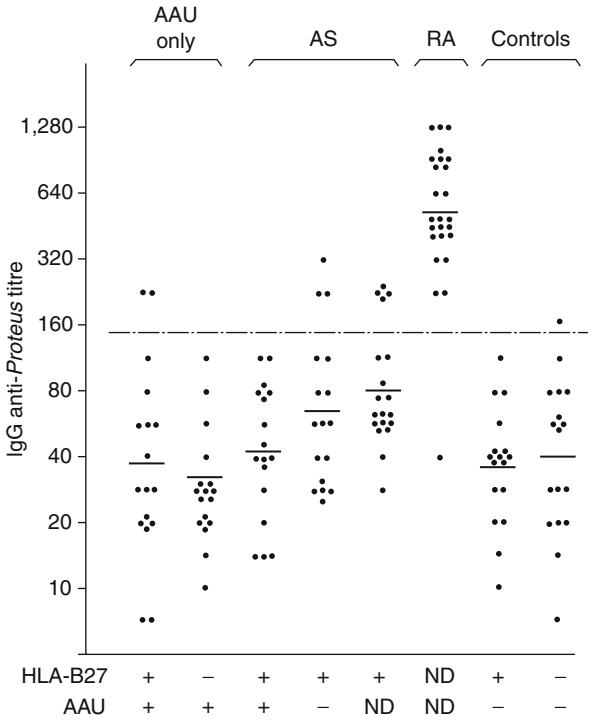

FIGURE 7.1 IgG anti-*Proteus mirabilis* antibody titres in sera of patients from groups 1–8 (Table 7.1). Each *dot* represents either a control subject or a patient (With permission from Blankenberg-Sprenkels et al. (1998))

IgG Antibodies to Proteus mirabilis

Patients with active rheumatoid arthritis patients showed significant elevations in IgG antibody titre when compared to either HLA-B27-positive controls ($t = 12.3$, $p < 001$) or when compared to HLA-B27-negative controls ($t = 10.6$, $p < 0.001$) (Fig. 7.1).

This antibody elevation in anti-*Proteus* titre was also significant when compared to all the other groups: HLA-B27-positive,

acute anterior uveitis–positive, ankylosing spondylitis–positive patients ($t=10.8$, $p<0.001$), HLA-B27-positive, acute anterior uveitis–positive, ankylosing spondylitis–negative patients ($t=9.79$, $p<0.001$), HLA-B27-positive, acute anterior uveitis–negative, ankylosing spondylitis–positive patients ($t=8.6$, $p<0.001$) and HLA-B27-positive patients with active ankylosing spondylitis ($t=8.8, p<0.001$).

IgA Antibodies to Klebsiella pneumoniae

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 ($t=6.27$, $p<0.001$) or HLA-B27-negative healthy controls ($t=11.37$, $p<0.001$) or in patients with rheumatoid arthritis ($t=11.36$, $p<0.001$) (Fig. 7.2).

Although no other ankylosing spondylitis or acute anterior uveitis showed anti-*Klebsiella* antibody titres above that of the patients with active ankylosing spondylitis, there were elevations above those seen in controls and patients with rheumatoid arthritis.

Patients who were HLA-B27-positive, acute anterior uveitis–positive and ankylosing spondylitis–positive and those who were HLA-B27-positive, acute anterior uveitis–negative and ankylosing spondylitis–positive had higher anti-*Klebsiella* antibody titres than patients who were HLA-B27-positive, anterior uveitis–positive, ankylosing spondylitis–negative but these differences were not statistically significant when corrected for number of groups examined. The titre of anti-*Klebsiella* antibodies could not be related to exacerbations or remissions in patients with acute anterior uveitis.

Furthermore HLA-B27-negative, acute anterior uveitis–positive and ankylosing spondylitis–negative patients had higher titres of anti-*Klebsiella* antibodies than either HLA-B27-positive controls ($t=2.16, p<0.05$) or HLA-B27-negative controls ($t=6.37, p<0.001$).

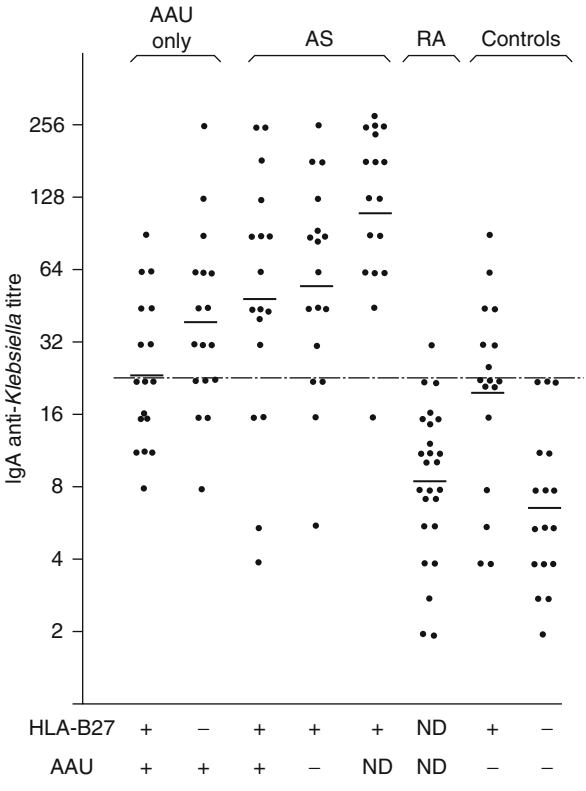

FIGURE 7.2 IgA anti-*Klebsiella pneumoniae* antibody titres in sera of patients from groups 1–8 (Table 7.1). Each *dot* represents either a control subject or a patient (With permission from Blankenberg-Sprenkels et al. (1998))

Clinical Implications

Rheumatoid arthritis patients had antibodies to *Proteus*, and ankylosing spondylitis and acute anterior uveitis patients had antibodies to *Klebsiella.* Each disease group was a specificity control for the other condition. It is clear that rheumatoid arthritis patients had antibodies against *Proteus* but not against

Klebsiella whilst the opposite was observed with the ankylosing spondylitis sera which had antibodies against *Klebsiella* but not against *Proteus*. Professor Bert Feltkamp said he was utterly surprised when his registrar told him that the London studies had picked both groups. The three original centres then refused to re-test the ankylosing spondylitis sera.

Here we are against the problem of simple, commercial test kits not being available to measure anti-*Klebsiella* or anti-*Proteus* antibodies which hampers clinical studies in patients with these diseases. The indirect immunofluorescence assay had been developed in house, in the Immunology Unit of King's College in the University of London by a very capable microbiologist, Dr. Mark Fielder who carried out the assays on the Dutch sera.

These results together with the immunofluorescence data from Toulouse and Brest confirm the previous work from our group in London, the studies from Dublin in Ireland and Newcastle in England that active rheumatoid arthritis patients have antibodies against a urinary pathogen.

An interesting observation was made in the tissue-typed controls. Those subjects who were HLA-B27-positive and deemed clinically healthy showed higher IgA anti-*Klebsiella* antibody titres than those healthy subjects typed as HLA-B27-negative. Similar observations have been made in England (Trull et al. 1983). In Finland, it was found that healthy HLA-B27-positive persons had elevated anti-*Klebsiella* titres of all immunoglobulin classes although only the IgM titres were statistically significantly increased (Toivanen et al. 1993). This may indicate that some subjects in the HLA-B27-positive 'healthy' control group are more likely to develop anti-*Klebsiella* antibodies. The higher anti-*Klebsiella* antibody titre in the HLA-B27 positive control group may also suggest a slow onset of ankylosing spondylitis but only longitudinal studies with close clinical follow-up and regular measurement of antibody levels to *Klebsiella pneumoniae* would resolve the problem.

The interesting question arises whether a similar observation occurs in healthy individuals who carry the 'shared

epitope' EQRRAA compared to individuals who are 'shared epitope' negative. Since approximately 35% of the general population in England, USA and the Netherlands carry the 'shared epitope', it would be relevant to determine whether such individuals have raised antibody levels against *Proteus mirabilis*.

If such antibodies are present, then they could act as early markers of rheumatoid arthritis. This would be similar to the situation of anti-CCP antibodies which are known to appear in the early stages of rheumatoid arthritis (Schellekens et al. 1998).

Conclusions

The data presented here show that there are specific and significant antibody elevations against *Proteus mirabilis* in rheumatoid arthritis patients from Amsterdam in the Netherlands. There are no antibody elevations against *Proteus mirabilis* in Dutch patients with acute anterior uveitis or ankylosing spondylitis. However, Dutch patients with ankylosing spondylitis or acute anterior uveitis have specific elevations in antibodies against *Klebsiella* microbes.

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