Chapter 10
Antibodies to *Proteus* in Rheumatoid Arthritis Patients from Southern Japan

Contents

The Japanese 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. These patients were being investigated in the 'Ankylosing Spondylitis Research Unit' of the Middlesex Hospital which had been set up in 1976 to study why up to 96% of ankylosing spondylitis with this disease possessed the HLA-B27 Major Histocompatibility group antigen whilst the frequency of this antigen in the general population of England or of the USA was only 8%.

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However, immunological studies in Japan showed that HLA-B27 was present in only 0.4% of the Japanese population, one of the lowest frequencies throughout the world.

Furthermore, ankylosing spondylitis, as a disease, was extremely rare in Japan, so rare that when the disease was observed, it was referred to orthopaedic surgeons because of their interests in backache and not to rheumatologists.

In 1993, I was approached by Professor Sinsuke Hukuda from Shiga University that a promising research worker and orthopaedic surgeon Dr. Yoshitaka Tani had been awarded a visiting scholarship from the Rheumatism Foundation of Japan to come to London and study antibodies to *Klebsiella* in Japanese ankylosing spondylitis patients. Since ankylosing spondylitis belonged to the orthopaedic medical community in Japan because of its rarity, it attracted the attention of this ambitious and hard working orthopaedic surgeon, Dr. Yoshitaka Tani as a research project. He collected all the ankylosing spondylitis patients in the southern parts of Japan and decided to determine whether they had elevated levels of antibodies to *Klebsiella* as had been reported in ankylosing spondylitis patients in England (Trull et al. 1983).

Dr. Yoshitaka Tani came to London with his ankylosing spondylitis sera and blood donor control sera. The ankylosing spondylitis and control sera were stored in a freezer, and he was told he should go back to Japan and bring back as well sera from active rheumatoid arthritis patients.

Active rheumatoid arthritis patients were defined as those having at least an erythrocyte sedimentation rate in excess of 30 mm/h (ESR > 30 mm/h) at the time when the blood sample was obtained. Then both ankylosing spondylitis sera and rheumatoid arthritis sera would be tested simultaneously against both *Klebsiella* and *Proteus* bacteria, under blind conditions in London. Each microbe would be acting as a disease control for the other condition.

Dr. Yoshitaka Tani returned to Japan and brought back to London a set of sera obtained from Japanese active rheumatoid arthritis patients from the Otsu area in southern Japan.

All the sera, from rheumatoid arthritis and ankylosing spondylitis patients as well as from the blood donors, were then tested under blind conditions against both *Klebsiella* and *Proteus* microorganisms, as well as against *Escherichia coli*.

Otsu: Location and History

Otsu is the capital city of the Shiga province in southern Japan. In 2006, Shiga was merged with Otsu and the size of the merged population was approximately 350,000.

In the years 667–672, Omiotsu Palace was founded in Otsu by Emperor Tenji. During the Edo period (1603–1867) when the Tokugawa shoguns were rulers of Japan, the poet Matsuo Basho frequented Otsu and took on apprentices.

On 11th May 1891, the 'Otsu incident' occurred, during a failed assassination attempt on Tsarevich Nicholas Alexandrovich of Russia.

The Shiga University is in the Shiga Prefecture of Japan. It comprises campuses from the cities of Otsu and Hikone.

The rheumatoid arthritis and ankylosing spondylitis patients came from the Shiga province in Japan.

Patients and Controls

Serum samples from 152 Japanese subjects were studied.

There were 30 patients with active rheumatoid arthritis as defined by the American Rheumatism Association criteria. There were 5 men and 25 women. The female to male ratio was 5:1. The mean age of the active rheumatoid arthritis patients was 51 years (range 23–70 years). The mean (±standard error) erythrocyte sedimentation rate of the active rheumatoid arthritis was 77.7 ± 5.9 mm/h. The mean C-reactive protein level of the active rheumatoid arthritis patients' mean (±standard error) was 40.2 ± 5.2 mg/l.

There were also 20 rheumatoid arthritis patients with probably active disease, 5 men and 15 women. The female to male ratio was 3:1. The mean age of the probably active rheumatoid

arthritis patients was 55 years (range 30–77 years). The mean (±standard error) erythrocyte sedimentation rate of the probably active rheumatoid arthritis patients was 38.3±9.4 mm/h. The mean (±standard error) C-reactive protein level of the probably active rheumatoid arthritis patients was 5.9±1.3 mg/l.

There were 29 sera from active ankylosing spondylitis patients selected according to the New York criteria. There were 27 men and 2 women and the male to female ratio was 13.5:1. The mean age of the active ankylosing spondylitis patients was 42 years (range 20–69 years). The mean (±standard error) erythrocyte sedimentation rate of the active ankylosing spondylitis patients was 49.0±9.7 mm/h. The mean (±standard error) C-reactive protein level of the active ankylosing spondylitis patients was 24.8±9.1 mg/l.

There were also 23 ankylosing spondylitis patients with inactive disease (21 men and 2 women) and the male to female ratio was 10.5:1.

The mean age of the inactive ankylosing spondylitis patients was 42 years (range 25–68 years). The mean (±standard error) erythrocyte sedimentation rate of the inactive ankylosing spondylitis was 12.2±2.1 mm/h. The mean (±standard error) C-reactive protein level of the inactive ankylosing spondylitis patients was 3.9±1.1 mg/l.

Furthermore, sera from 50 healthy controls (25 men and 25 women) were supplied by the Red Cross Blood Centre in Otsu, Japan.

Active patients were deemed to be those who had an erythrocyte sedimentation rate greater than 20 mm/h and a serum C-reactive protein level above 10 mg/l.

Probably active patients were considered those with at least one of these variables elevated.

Inactive patients were those with an erythrocyte sedimentation rate below 15 mm/h and a serum C-reactive protein level below 10 mg/l.

All ankylosing spondylitis patients except one were HLA-B27 positive.

Some 93.3% of the active rheumatoid arthritis patients possessed either HLA-DR1 or HLA-DR4. Furthermore, some 40% of the rheumatoid arthritis patients had both HLA-DR1 and DR4.

Control subjects were not tissue typed.

Results of ELISA Studies

ELISA studies were carried out as previously described against three different microbes: *Proteus mirabilis*, *Escherichia coli* and *Klebsiella pneumoniae*. All assays were carried out under code so that the status of each serum sample under investigation was not known to the tester.

Patients with active rheumatoid arthritis showed elevated levels of IgG antibodies against *Proteus mirabilis* when compared to controls and this difference was statistically significant ($t = 14.10, p < 0.001$) (Fig. 10.1).

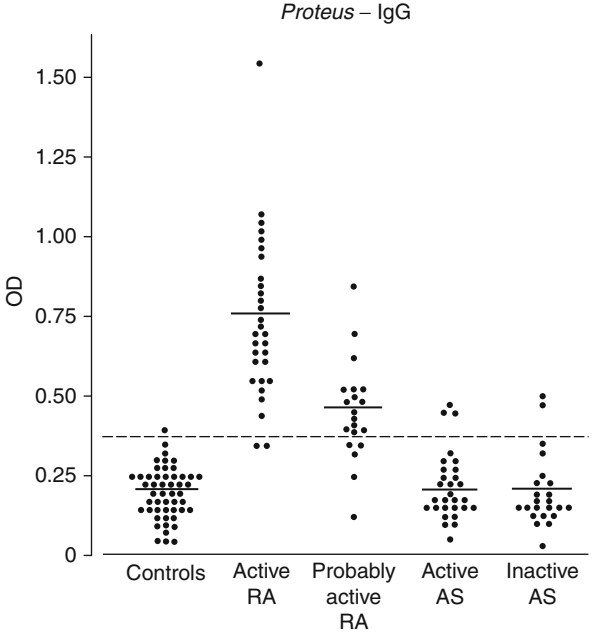

FIGURE 10.1 IgG antibody titres against *Proteus mirabilis* in controls, active rheumatoid arthritis patients, probably active rheumatoid arthritis patients, active ankylosing spondylitis patients and inactive ankylosing spondylitis patients. The *broken line* represents 95% confidence limit of the distribution of the controls (bars = means). Each *dot* represents either a control subject or a patient (Reprinted from Tani et al. (1997), with kind permission)

Similar results were seen in probably active rheumatoid arthritis patients when compared to controls, and this difference was also statistically significant ($t = 8.30, p < 0.001$).

Active rheumatoid arthritis patients also had elevated levels of IgM antibodies against *Proteus mirabilis* when compared to controls and again this difference was statistically significant ($t = 3.72, p < 0.001$).

Probably active rheumatoid arthritis patients did not exhibit IgM antibody elevations against *Proteus mirabilis*.

There were no IgG, IgA or IgM antibody elevations in active or probably active rheumatoid arthritis patients when tested with *Klebsiella pneumoniae* or *Escherichia coli*.

Ankylosing spondylitis patients with active disease had elevated levels of IgA antibodies against *Klebsiella pneumoniae* and this difference was statistically significant when compared to controls ($t = 5.72, p < 0.001$) (Fig. 10.2).

Inactive ankylosing spondylitis patients showed no elevations in IgA, IgG or IgM antibodies against *Klebsiella pneumoniae*.

Active and inactive ankylosing spondylitis had no elevations in IgG or IgM antibodies against *Klebsiella pneumoniae*.

There were no IgG, IgA or IgM antibody elevations in either active or inactive ankylosing spondylitis patients against *Proteus mirabilis* or *Escherichia coli*.

Discussion and Conclusions

The data presented here show that there are specific and significant antibody elevations against *Proteus mirabilis* in rheumatoid arthritis patients from Otsu in southern Japan.

These results would appear to be specific, since Japanese ankylosing spondylitis had antibody elevations against *Klebsiella pneumoniae* but not against *Proteus mirabilis* whilst the reverse was observed with Japanese rheumatoid arthritis patients.

This would appear to be the first time that Japanese rheumatoid arthritis patients have been found to have elevated levels of specific antibodies against the urinary microbe *Proteus mirabilis* (Tani et al. 1997).

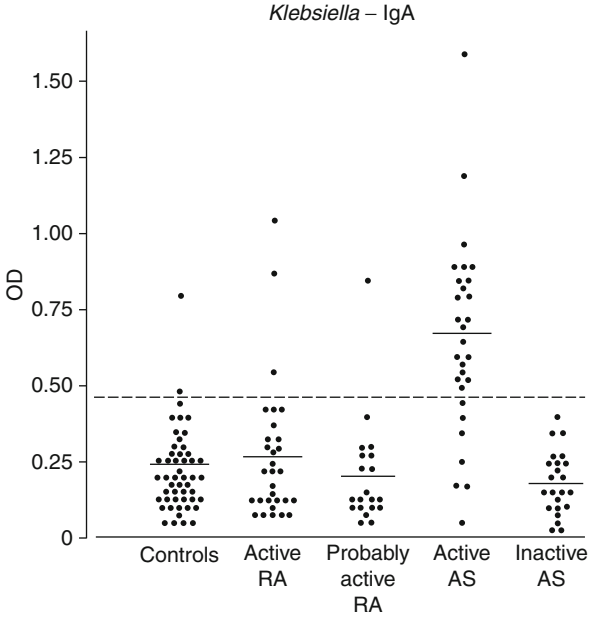

FIGURE 10.2 IgA antibody titres against *Klebsiella pneumoniae* in controls, active rheumatoid arthritis patients, probably active rheumatoid arthritis patients, active ankylosing spondylitis patients and inactive ankylosing spondylitis patients. The *broken line* represents 95% confidence limit of the distribution of the controls (bars = means). Each *dot* represents either a control subject or a patient (Reprinted from Tani et al. (1997), with kind permission)

However, similar Japanese studies have shown that antibodies to 'common enterobacterial antigens' are present in the serum and synovial fluids of patients with rheumatoid arthritis (Aoki et al. 1996).

These results would seem to suggest that the Gram-negative microbe *Proteus mirabilis* is somehow involved in the onset and pathogenesis of rheumatoid arthritis at least in Japanese patients coming from Otsu and surrounding areas. Studies from the USA and Canada indicate that antibodies to

Proteus mirabilis can be detected in the early stages of rheumatoid arthritis (Newkirk et al. 2005).

The results from these Japanese patients appear to be similar to those obtained from rheumatoid arthritis studies in European and American patients.

The general tentative conclusion can be proposed that *Proteus mirabilis* is involved in the causation of rheumatoid arthritis in populations throughout the world.

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