Chapter 6
Antibodies to *Proteus* in Rheumatoid Arthritis Patients from Brest and Toulouse in France

Contents

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Brest and Toulouse: Introduction

In the early 1990s, Professor Pierre Youinou from the University of Brest in France carried out collaborative studies on lymphocytes with Professor Ivan Roitt, in the Department of Immunology at the Middlesex Hospital in London. He came to know the work being carried out by the King's College Immunology group on *Proteus* antibodies in English patients and asked whether this was a general observation which applied to rheumatoid arthritis patients in different countries. He was interested in the question if similar observations could be made on French patients suffering from rheumatoid arthritis. He said that he could obtain sera from rheumatoid arthritis patients in Brest and also from a colleague Dr. A. Cantagrel in Toulouse, in south-western France and would provide control sera from the Blood Bank in Brest. However, he insisted that he would code the sera himself in France before they were dispatched to the UK. The studies would be carried out in London, results then sent back to France, decoded and the appropriate calculations carried out.

It was decided to compare rheumatoid arthritis patients from Brest and Toulouse to a set of control sera supplied by the Blood Bank in Brest.

The antibody studies were carried out by two different techniques, ELISA and immunofluorescence, on the Brest rheumatoid arthritis patients. The sera from patients with rheumatoid arthritis from Toulouse were tested only by immunofluorescence.

Brest: Location and History

Brest is located at the western tip of France and has a wide bay which makes it a natural harbour. The town has a population of 150,000 but provides services to about 1 million inhabitants of western Brittany. The city was almost completely destroyed during the Second World War because it was an important submarine base but has been largely rebuilt.

Brittany was an independent Duchy in the Middle Ages but was brought to the French crown as a dowry by the marriage of Anne de Bretagne to the French King.

Some 200,000 inhabitants of Brittany speak Breton, a Celtic language related to Cornish and Welsh, and there are strong cultural links with Wales, Galicia and Ireland.

Toulouse: Location and History

Toulouse is situated in south-western France on the banks of the Garonne river. It has been inhabited for almost 3,000 years, and during Roman times, it was known as Tolosa. It has a population of over 1 million. Its university is one of the oldest in Europe having been founded in 1229. During the early Middle Ages, it was the centre of a Visigothic empire stretching from southern Spain to northern France. More recently, it was the capital of the Languedoc region.

Hopital de Rangueil is situated in a university campus in Toulouse where Dr. A. Cantagrel was involved in rheumatological services. He provided the sera from 15 active rheumatoid arthritis patients to be studied by immunofluorescence for the presence of anti-*Proteus* antibodies.

Patients and Controls

All the rheumatoid arthritis sera from both Brest and Toulouse were selected on the basis of clinical assessment of disease activity by the attending physicians and an elevated erythrocyte sedimentation rate (ESR) greater than 15 mm/h.

Sera from Brest

Sera from 50 tissue-typed, active rheumatoid arthritis patients and 49 healthy controls were obtained from the Laboratoire d'Immunologie et de Rhumatologie, Centre Hospitalier

Régional et Universitaire (CHRU) from Brest in France. Patients were tissue typed by the micro-cyto-toxicity assay of Terasaki using standard reagents (France-Transplant). The characteristics of the Brest patients and controls were as follows. The mean age of the rheumatoid arthritis group was 60 years (range 14–84). In the rheumatoid arthritis patients, the mean erythrocyte sedimentation rate (± standard error) was 70.0 ± 3.0 mm/h. The female/male ratio in the rheumatoid arthritis patients from Brest was 2.3. The female/male ratio in the healthy controls was 2.8. Erythrocyte sedimentation rates were not measured in the controls. The percentage of rheumatoid arthritis–associated tissue types (DRB1*0101 (DR1) and DR4 combined) was 60%. Additionally, 8% of the rheumatoid arthritis patients carried HLA-B27. Control subjects were not tissue typed.

Sera from Toulouse

Sera were also collected from 15 patients with active rheumatoid arthritis attending the Rheumatology Department of the Rangueil Hospital in Toulouse. The rheumatoid arthritis patients from Toulouse were not tissue typed and the sera were used only for immunofluorescence studies.

ELISA Studies with Three Bacteria: *Proteus mirabilis*, *Escherichia coli* and *Salmonella typhimurium*

The ELISA studies, involving three different bacteria, *Proteus mirabilis*, *Escherichia coli* and *Salmonella typhimurium*, were carried out only on the rheumatoid arthritis sera from Brest, as previously described (Fielder et al. 1995).

The active rheumatoid arthritis patients from Brest had an elevated level mean (± standard error) of IgG *Proteus* antibodies of 1.156 ± 0.068 OD units when compared to the level in control subjects of 0.730 ± 0.160 OD units and this difference was statistically significant ($t = 4.24$, $p < 0.001$) (Fig. 6.1).

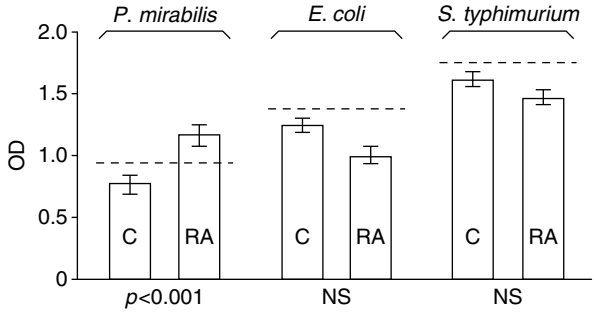

FIGURE 6.1 Antibody titres (mean ± standard error) for IgG in 49 controls (C) and 50 active rheumatoid arthritis patients (RA) from Brest when tested by ELISA, under code, against *Proteus mirabilis*, *Escherichia coli* and *Salmonella typhimurium*. (*Dotted line* indicates 95% confidence limits for mean of controls – one-tailed test; OD optical density) (Reprinted from Fielder et al. (1995), with permission from Springer Science + Business Media)

However, there was no significant elevation in IgG antibody titres in rheumatoid arthritis patients from Brest against *Escherichia coli* or *Salmonella typhimurium*.

Serum C-reactive protein level was measured in the Brest patients and controls by the radial immunodiffusion method of Mancini (Mancini et al. 1965).

The mean (± standard error) C-reactive protein level in the rheumatoid arthritis patients was 57.6 ± 5.0 mg/l, whilst in the control subjects, it was 18.7 ± 5.7 mg/l, and this difference was statistically significant ($t = 5.12$, $p < 0.001$).

Indirect Immunofluorescence Studies with *Proteus* in Rheumatoid Arthritis Patients from Brest and Toulouse

Serum anti-*Proteus* antibodies in 50 rheumatoid arthritis patients from Brest and 15 rheumatoid arthritis patients from Toulouse were measured by indirect immunofluorescence as previously described. The results were scored as

follows: A score of zero was given for no fluorescence at a serum dilution of 1/10, whilst fluorescence at 1/10 scored 1, fluorescence at 1/20 scored 2, fluorescence at 1/40 scored 3, fluorescence at 1/80 scored 4 and so on up to a value of 1/1280 which scored 8. The results were described as $\log_2$ dilution units. The studies were again carried out under code in that the person doing the assay did not know which sera came from rheumatoid arthritis patients and which came from control subjects.

The mean (± standard error) anti-*Proteus* antibody titre in the rheumatoid arthritis patients from Brest was 6.51 ± 0.16 whilst the mean (± standard error) in the blood donors was 3.70 ± 0.16 and this difference was statistically significant ($t = 8.96$, $p < 0.001$) (Fig. 6.2).

The mean (± standard error) anti-*Proteus* antibody titre in the rheumatoid arthritis patients from Toulouse was 6.30 ± 0.34 and this difference was again statistically significant when compared to the blood donors from Brest ($t = 4.96$, $p < 0.001$) (Fig. 6.2).

These results suggest that French rheumatoid arthritis patients, at least from Brest and Toulouse, also have specific elevations in antibody levels against *Proteus* microbes (Fielder et al. 1994).

The indirect immunofluorescence studies confirmed the results obtained by the ELISA technique in that French rheumatoid arthritis patients have elevated levels of antibodies to *Proteus* when compared to their respective national blood donor controls (Fielder et al. 1995).

Clinical Implications

The results by using two different techniques show that rheumatoid arthritis patients from Brest have specific antibody elevations against the urinary microbe *Proteus mirabilis* but not against *Escherichia coli* and *Salmonella typhimurium*. These results together with the immunofluorescence data from Toulouse confirm the previous studies from London, Dublin in Ireland,

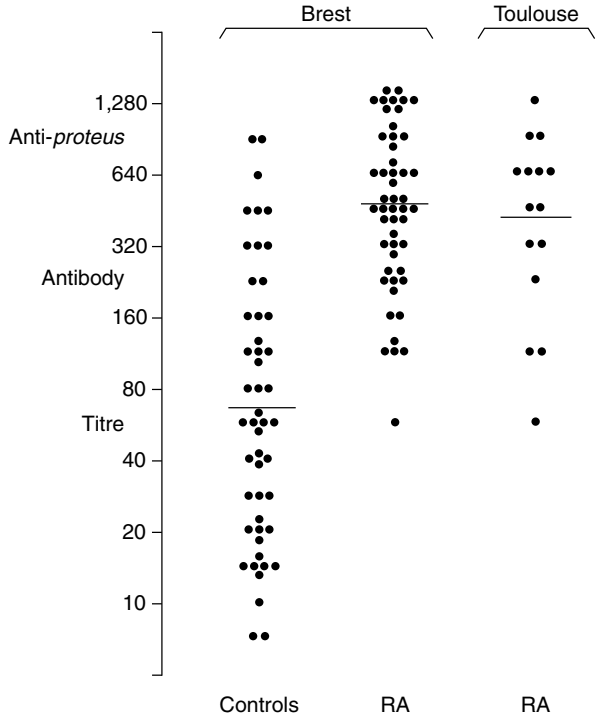

FIGURE 6.2 Antibody titres (bar = mean) for IgG in 49 controls and 50 active rheumatoid arthritis patients from Brest and 15 rheumatoid arthritis patients from Toulouse when tested by indirect immunofluorescence assay against *Proteus mirabilis*. Each *dot* represents the serum of either a rheumatoid arthritis patient or a blood donor subject (Reprinted from Fielder et al. (1995), with permission from Springer Science + Business Media)

Newcastle in England and Bermuda as well as northern Hertfordshire in that active rheumatoid arthritis patients have antibodies against a specific urinary pathogen *Proteus mirabilis*.

Furthermore, the ELISA studies show that this elevation is not present against *Escherichia coli*, a microbe involved frequently in causing cystitis especially in women.

References

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