Chapter 8
Anti-*Proteus* Antibodies in Norwegian Rheumatoid Arthritis Patients Following a Lactovegetarian Diet

Contents

Norway: An Introduction

Norway is a Nordic country occupying the western part of the Scandinavian peninsula. It is a country of famous fjords and has a population of about five million.

It was united into one kingdom by Harald the First of Norway in the early 800s which was then followed by two

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centuries of Viking raids into various parts of Europe including England, Scotland and Ireland.

Colonies were also established in Iceland, Greenland, North America also known as Vinland and Eastern Europe.

In 1397, it was absorbed into a union with Denmark. Following the destruction of the kingdom of Denmark–Norway during the Napoleonic wars, it merged with Sweden. However, in 1905, following a plebiscite, Norway became an independent country.

It is a country with a high life-expectancy and rheumatoid arthritis is a common disease, as in all countries where there is a high proportion of the population over the age of 50 years.

Patient Population and Diet Study

It has been suggested that *Proteus mirabilis*, a microbe known to cause urinary tract infections, might be relevant in the study of the pathogenesis of rheumatoid arthritis.

A Norwegian group, led by Dr. J. Kjeldsen-Kragh, examined the association between disease activity in rheumatoid arthritis patients during a controlled clinical trial of fasting and a 1-year vegetarian diet (Kjeldsen-Kragh et al. 1991). He had heard about the work in London about antibodies to *Proteus* in rheumatoid arthritis patients and asked us to cooperate in a coded study.

Fasting is an effective method of treating rheumatoid arthritis, but most patients relapse on reintroduction of food.

The effect of fasting followed by 1 year of a vegetarian diet was assessed in a randomised, single-blind control trial. Briefly 53 rheumatoid arthritis patients were studied: 27 rheumatoid arthritis patients were randomly assigned to the diet group and 26 rheumatoid arthritis patients were assigned to the control group. The diet group were invited to a 4-week stay at a health farm. After an initial 7–10 day subtotal fast, they were put on an individually adjusted gluten-free vegan diet for 3.5 months. The food was then gradually changed to a lactovegetarian diet for the remainder of the study. The quantity of fluid intake was not measured.

A control group of 26 rheumatoid arthritis patients stayed for 4 weeks at a convalescent home but ate their ordinary, omnivorous diet throughout the whole study period.

Ten patients were withdrawn from the treatment group and nine from the control group during the course of the study.

After 4 weeks at the health farm, the diet group showed a significant improvement in the number of tender joints, the Ritchie articular index, number of swollen joints, pain score, duration of early morning stiffness, erythrocyte sedimentation rate, C-reactive protein levels, white blood cell count and a health assessment questionnaire score.

In the control group, only the pain assessment score improved.

Blood samples were collected at baseline, and after 1, 4, 7, 10 and 13 months. Serum samples were stored at –20°C until required for analysis.

The rheumatoid arthritis patients in the vegetarian group were subsequently divided into diet responders and diet non-responders, depending on the clinical results following the diet.

Briefly, a rheumatoid arthritis patient was categorised as a diet responder if there had been a substantial improvement in three of six core variables: number of swollen joints, functional disability, pain score, number of tender joints, patient's global assessment and erythrocyte sedimentation rate. The distinction into diet responder and diet non-responder was made before the antibody analyses were carried out.

Antibody analyses against *Proteus mirabilis* and *Escherichia coli* were carried out in all three groups of rheumatoid arthritis patients: diet responders, diet non-responders and controls.

Serum samples were coded in Norway, then sent to the Immunology Unit at King's College in London where the antibody assays were carried out. The results were then sent back to Norway where they were decoded.

In the diet responder group, the benefits were still present after 1 year. The evaluation of the whole course showed significant improvements for the diet in all measured indices.

Disease Activity Index

The relationship between disease activity and the levels of antibodies against *Proteus mirabilis* and *Escherichia coli* was investigated in all rheumatoid arthritis patients. The disease activity at each clinical examination was graded by means of a Stoke disease activity index, slightly modified by replacing the proximal interphalangeal joint synovitis score with the total number of swollen joints (Davis et al. 1990).

Determination of *Proteus mirabilis* and *Escherichia coli* Antibody Titres

ELISA determinations on whole bacteria were carried out as previously described. The statistical analyses were carried out as follows:

To test for within-group differences, the Wilcoxon signed rank test was used. Kruskal–Wallis test was used for comparison of three groups. As a post hoc test for the latter comparisons, the Mann–Whitney test was used. Correlations were studied by Spearman's rank correlation analysis. For the patients who were prematurely withdrawn from the trial, values were extrapolated as described (Kjeldsen-Kragh et al. 1991).

In all tests, except for post hoc analyses, p-values less than 0.05 were considered as significant.

As the post hoc analyses involved three comparisons, only those p-values less than 0.0167 were regarded as significant for these tests.

Results of the Antibody Assays in Rheumatoid Arthritis Patients

Patients who followed the lactovegetarian diet had a significant reduction in mean anti-*Proteus* antibody titre at 4, 7, 10 and 13 months (Table 8.1).

Table 8.1 Decrease from baseline with time for anti-*Proteus mirabilis* and anti-*Escherichia coli* IgG ($\log_2$ dilution units). Statistical significance compared to baseline

	Time elapsed after baseline measurements		
	7 months	**10 months**	**13 months**
Anti-*Proteus* antibody	$p<0.01$	$p<0.01$	$p<0.01$
Diet responders	1.417	1.125	1.042
Diet non-responders	0.467	0.200	0.233
Omnivores	–0.077	0.346	0.385
Anti-*E.coli* antibody	NS	NS	NS
Diet responders	0.058	0.025	0.089
Diet non-responders	–0.035	–0.073	–0.062
Omnivores	0.004	–0.006	–0.013

NS not significant

No significant change in mean antibody titre was observed in the omnivorous patients.

In the rheumatoid arthritis patients who eventually were categorised as 'diet responders', the mean anti-*Proteus* antibody titre decreased significantly compared with baseline after 1, 4, 7 and 10 months. At all these times, the antibody titres were least for the diet responders; post hoc analysis revealed that the change from baseline was significantly greater in the diet responders compared to the rheumatoid arthritis patients in the control, omnivorous group.

Changes from baseline of the modified Stoke disease activity index were examined with the changes from baseline with the anti-*Proteus mirabilis* and the anti-*Escherichia coli* antibody levels.

Disease improvement correlated significantly with the anti-Proteus *mirabilis* titres ($r=0.31$, $p<0.001$) (Fig. 8.1) but not with the changes in anti-*Escherichia coli* antibody levels ($r=-0.012$, not significant).

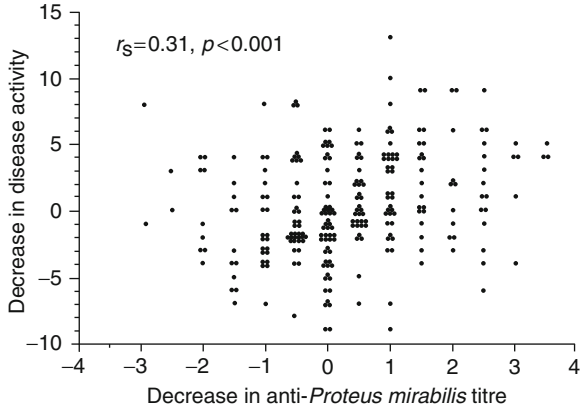

FIGURE 8.1 Scattergram of the decrease from baseline in anti-*Proteus* titres in the rheumatoid arthritis patients versus the decrease from baseline in the modified Stoke disease activity index. p-value by Spearman rank correlation (r_s = correlation coefficient) (Reprinted from Kjeldsen-Kragh et al. (1995), with permission from BMJ Publishing Group)

The lack of a significant correlation with anti-*Escherichia coli* antibodies in Norwegian patients with rheumatoid arthritis is in agreement with the studies on Bermudian and English patients from Stevenage with rheumatoid arthritis who also showed no association with the cystitis causing bladder microbe, namely *Escherichia coli.*

All statistical analyses were also performed on the original data that had not been extrapolated to account for withdrawn patients. These analyses gave almost the same results as the analyses for the extrapolated data.

Discussion

This is a comprehensive longitudinal study of anti-*Proteus mirabilis* antibodies in rheumatoid arthritis patients following dietary therapy. A significant decrease in anti-*Proteus mirabilis* antibodies was found in the diet responders

compared with the diet non-responders and omnivores (Kjeldsen-Kragh et al. 1995).

The decrease in *Proteus* antibody titres further substantiates the suggestion that these antibodies are of central importance in the pathogenesis of rheumatoid arthritis.

Fasting and vegetarian diets give rise to significant changes in the gastro-intestinal bacterial flora (Midvedt et al. 1990). Furthermore, uncooked vegan diets alter the microbial bowel flora (Peltonen et al. 1992).

Peltonen's group have analysed the faecal flora collected from rheumatoid arthritis patients who participated in the controlled clinical trial of fasting and 1-year vegetarian diet (Peltonen et al. 1994).

Significant differences in the faecal flora were observed between samples obtained at times which coincided with pronounced clinical improvement and samples obtained at times of low or no improvement. However, it remains to be demonstrated that these differences were the result of a decrease in the amount of *Proteus mirabilis* in the faeces, which in turn might reduce the absorption of *Proteus* antigens from the gut.

A shift from an omnivorous diet to a vegetarian diet has a profound influence on the composition of the urine. Vegetarians may have an increased fluid intake although this was not specifically investigated in this study. High fluid intake has been thought to be beneficial in patients with urinary tract infections and this could explain the decreased anti-*Proteus mirabilis* antibody titres in rheumatoid arthritis patients on lactovegetarian diets. Furthermore, some plant products like cranberry juice are frequently used in the treatment of urinary tract infections.

Vegetarians have a greater urinary excretion of lignans and phytooestrogens metabolites than omnivores. Some of these substances are known to possess antibacterial activity in vitro (Ito et al. 1982).

A positive correlation between C-reactive protein levels and the level of anti-*Proteus* antibody titres has been reported by the London group and the Newcastle group.

In the present study, a significant correlation was found between the changes in anti-*Proteus* antibody titres and the changes in a disease activity index composed of five different variables following a lactovegetarian diet. Clearly further studies are indicated to determine the role of anti-*Proteus* antibodies in the pathogenesis of rheumatoid arthritis.

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