ANTI-PROTEUS ANTIBODIES AND PROTEUS ORGANISMS IN RHEUMATOID ARTHRITIS: A CLINICAL STUDY

J. McDonagh*, J. Gray†, H. Sykes* D. J. Walker*, A. J. Bint† and C. M. Deighton

Departments of *Rheumatology and †Microbiology, Royal Victoria Infirmary, Newcastle-upon-Tyne
NE1 4LP

SUMMARY

We have studied anti-Proteus antibodies (APA), isolation of Proteus, and their relation to various measures of RA disease activity. Seventy RA patients with a CRP>10 mg/l had higher APA titres than 17 RA patients with CRP<10 mg/l (P = 0.006), and 36 non-RA controls (P = 0.003). However, in a cross-sectional study of the RA group, there was no correlation between APA and a number of clinical and laboratory measures of disease activity, including the CRP and Stoke RA activity index. A longitudinal study showed no correlation between changes in these measures of disease activity and change in APA titre. We were unable to isolate Proteus in the urine or faeces of RA patients more frequently than controls, and the isolation of Proteus did not correlate with serum APA titres. Urinary APA was present in equal frequencies in RA and non-RA patients. NSAIDs, DMARDs and steroids did not appear to influence APA titres in the RA group. These results suggest that APA may act as an acute phase protein, distinct from CRP, but not correlating with RA disease activity in its broadest context. The fact that the antibody we are measuring binds to Proteus may be irrelevant, and the study does not support a role for Proteus in RA.

KEY WORDS: Anti-Proteus antibodies, Rheumatoid arthritis, Disease activity, Stoke index, Urine, Faeces, Drugs, Acute phase protein.

We are one of three groups to independently demonstrate that anti-Proteus antibodies (APA) are elevated during active phases of RA [1-3]. However, our criteria for measuring disease activity has rested exclusively with an elevation of CRP greater than 10 mg/l. This has understandably been criticized, as CRP may be elevated for reasons other than disease activity [4]. There was clearly a need to address this area taking RA disease activity in a broader context. Furthermore, we wanted to attempt to isolate Proteus organisms and anti-Proteus antibodies in the urine of patients with RA, and determine whether this could be achieved more frequently in patients with active than both inactive RA and non-RA controls. Finally, we wanted to determine whether patient medication might account for the phenomenon of elevated APAs, as has been suggested for Clostridium perfringens antibodies [5]. We therefore embarked on cross-sectional and longitudinal clinical studies to address these areas.

METHOD

I. Cross-sectional study

(a) Patients clinical information

Patients with RA fulfilling the 1958 ARA criteria for classical and definite disease [6] were ascertained from inpatient wards and outpatient clinics, along with a control group of patients with non-inflammatory disorders such as mechanical back pain and OA. Each patient was interviewed using a standard questionnaire, obtaining the following information, where appropriate to their disease status: duration of morning stiffness, a pain score on a visual analogue scale (0-100), Ritchie index [7], grip strength with a standard cuff at 30 mmHg, assessment of the presence of proximal interphalangeal synovitis, scoring each finger and thumb joint on a four point scale (0 = nil, 1 = possible, 2 = definite, 3 = gross). Blood was taken for: ESR, full blood count, alkaline phosphatase, immunoglobulins (nephelometry, Beckman Array) and CRP (enzyme immunoassay: Emit, Syva U.K., Maidenhead).

(b) Anti-Proteus antibodies

The indirect immunofluorescence technique for the detection of serum APAs has been described previously [1]. Briefly, using National Collection of Type Cultures (NCTC, London) strains of Proteus mirabilis (NCTC 11938), an overnight Todd-Hewitt broth (Oxoid Ltd) subculture of the organism was washed three times in phosphate buffered saline (PBS) in order to try and avoid the possibility of the APA detecting bovine IgG adsorbed to the organism from the Todd-Hewitt broth. This was then centrifuged, resuspended in PBS and then fixed on to 8-well slides and reacted with serial two-fold dilutions of patients’ sera starting at 1/10, followed by fluorescein-labelled sheep anti-human immunoglobulin (Wellcome Diagnostics, Dartford). Positive and negative controls were included in each run, the former being serum of a 87-yr-old female non-RA patient with a P. mirabilis septicemia secondary to pyelonephritis. All sera were tested in duplicate and under code. Serial two-fold dilutions of urine were tested for APA, using the same method.

Specificity of the test was confirmed in preliminary work where sera from seven patients with Gram negative septicemia (including two with P. mirabilis) and 20 patients with active RA were tested by immunofluorescence against six species of Enterobacteriaceae (Escherichia coli, Proteus vulgaris, P. mirabilis, Kleb-
TABLE I
Spearman rank correlation coefficients between antibody titres and various measures of disease activity in RA patients in cross-sectional and longitudinal studies

<table>
<thead>
<tr>
<th>Disease activity variable</th>
<th>Cross-section study (n = 87 RA patients)</th>
<th>Longitudinal study (n = 43 RA patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>CRP</td>
<td>0.02</td>
<td>0.42</td>
</tr>
<tr>
<td>ESR</td>
<td>0.11</td>
<td>0.19</td>
</tr>
<tr>
<td>Morning stiffness</td>
<td>-0.04</td>
<td>0.38</td>
</tr>
<tr>
<td>Ritchie index</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>Grip strength</td>
<td>0.07</td>
<td>0.30</td>
</tr>
<tr>
<td>Pain VAS</td>
<td>0.08</td>
<td>0.25</td>
</tr>
<tr>
<td>HAQ score</td>
<td>-0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>0.02</td>
<td>0.42</td>
</tr>
<tr>
<td>Platelets</td>
<td>-0.04</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Abbreviations: VAS, visual analogue scale; HAQ, Health Assessment Questionnaire.

siella pneumoniae, Salmonella enterica, Salmonella sonnet). In a further study, immunofluorescence was removed from APA positive RA sera by prior absorption with P. mirabilis (data not shown).

(c) Culture methods
Clean catch urine samples were cultured on the day of receipt in the laboratory. Faecal samples were also obtained from some patients, and where there was a clinical indication for obtaining SF, a sample was sent for culture.

Urine. Clean catch urine specimens were cultured by a standard laboratory method (in order to determine whether bacteria other than Proteus were present in the specimen) and by an enrichment technique selective for Proteus. In the standard method 10 μl of urine was plated on to blood agar and MacConkey agar and incubated in air at 37°C for 24 h. For selective enrichment, 1 ml of urine was inoculated into 10 ml Tryptone soya broth (Oxoid Ltd., Basingstoke) containing polymyxin B 50 mg/l and vancomycin 8 mg/l. After incubation at 37°C, isolates were identified according to standard laboratory procedures [10]. In preliminary experiments, the selective enrichment technique was shown to reliably detect less than five organisms per ml of urine.

Faeces. A pea-sized sample of faeces was inoculated into 10 ml of Proteus selective enrichment broth and vortexed vigorously before incubation and subculture as described for urine specimens.

Synovial fluid. SF was cultured on chocolate agar (incubated in 5% CO₂) and blood agar (incubated anaerobically) at 37°C for 4 days. A further 1 ml of SF was added to 10 ml of Todd–Hewitt broth (Oxoid Ltd.), incubated at 37°C for 48 h, then subcultured onto chocolate agar for incubation for a further 4 h at 37°C in 5% CO₂.

2. Longitudinal study
Forty-three of the RA patients in the cross-sectional study were reviewed approximately 3 months after the original interview and examination. A similar laboratory assessment to the first one was made, with serum and urine samples obtained.

3. Statistical analysis
Firstly the population was divided into three groups: active RA (CRP > 10 mg/l), inactive RA (CRP ≤ 10 mg/l), and non-RA, to attempt to confirm our previous finding of higher APA in the first group compared with the second and third, using the Mann–Whitney U test. Correlations between APAs and the various clinical and laboratory measures of disease activity were assessed and the proximal interphalangeal synovitis score, ESR, duration of morning stiffness, CRP and Ritchie index score were then combined to form the Stoke activity index [9]. Changes in the various measures of disease activity between assessments in the longitudinal study of RA patients were addressed to determine correlations with changes in anti-Proteus antibody titre. All correlations were calculated using the Spearman rank correlation coefficient.

The isolation of Proteus organisms in the urine and faeces of RA patients was compared with the non-RA controls using χ². The Mann–Whitney U test was used to determine whether the isolation of Proteus in urine and faeces was associated with higher levels of APA. The detection of APAs in the urine of RA and non-RA patients was assessed using χ². The same test was used to decide whether various categories of medication.
TABLE III

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Anti-Proteus antibody titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1/10</td>
</tr>
<tr>
<td>Nil (%)</td>
<td>6.3</td>
</tr>
<tr>
<td>Proteus (%) alone (n = 3)</td>
<td>0.0</td>
</tr>
<tr>
<td>Proteus (%) and other organisms (n = 13)</td>
<td>0.0</td>
</tr>
<tr>
<td>E. coli (%) alone (n = 5)</td>
<td>0.0</td>
</tr>
<tr>
<td>Others (%) (n = 18)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

(e.g. NSAIDs, analgesics, sulphasalazine, other second-line agents, prednisolone and antibiotics) were associated with differences in APA titre. All statistics were performed on the Statistical Package for the Social Sciences (SPSSx).

RESULTS

Eighty-seven patients with classical or definite RA were ascertained. Seventy-one (81.6%) were female. Their median age was 65 yr (range 19 to 80 yr) with a median disease duration of 12 yr (range 1 to 40 yr). There were 36 non-inflammatory arthritis controls. Twenty-three (63.9%) were female, with a median age of 60 yr (range 25 to 75 yr).

Of the 87 RA patients, 70 had a CRP > 10 mg/l. Their median APA was 1/40 (range <1/10 to 1/1280). This was significantly higher than in the 17 RA patients with CRP ≤ 10 mg/l (median 1/20, range 1/10 to 1/80, P = 0.006) and the non-RA controls (median 1/20, range <1/10 to 1/40, P = 0.003). There was no significant difference in the distribution of APA in the latter two groups (P = 0.56). There was also no correlation between total serum immunoglobulins and the serum APA titre (Spearman rank correlation coefficient was 0.2, P = 0.17).

The distribution of the Stoke disease activity index and APAs in the RA patients is shown in Fig. 1. The Spearman rank correlation coefficient was 0.007 (P = 0.48).

Correlation coefficients for various measures of disease activity and APAs for the cross-sectional study are shown in Table I. This table also shows the data for the 43 RA patients assessed in the longitudinal study, showing correlation coefficients between the disease activity variable and APA. There were no significant correlations between APA and disease activity measures in either the cross-sectional or longitudinal studies.

Urine samples were available from 71 (81.6%) of the patients, and 29 (67.4%) of the non-RA controls. The results of urine culture are shown in Table II. There were no significant differences in the isolation of Proteus of other micro-organisms in the three patient groups. Proteus was isolated from faeces samples from nine out of 30 RA patients (30%), and three out of 12 non-RA samples (25%, χ² is not significant). SF from 12 RA patients and one non-RA control revealed no growth.

The distribution of APAs for categories of isolates in urine in the RA patients is shown in Table III. There was no evidence to support the suggestion that the isolation of Proteus from urine had any bearing on the serum APA titre. Similarly, there was no association between serum APA titres and faecal carriage of Proteus in RA patients (median 1/20 in both carriers and non-carriers).

APAs were detected in undiluted urine in 41.2% of the RA patients, compared with 45.8% of the controls. Within the RA group, 41.7% of the 36 patients with a CRP > 10 mg/l had detectable urinary APA, compared with 40.0% of those with CRP ≤ 10 mg/l. Only three patients, two from the RA group (one with CRP > 10 mg/l, one with CRP > 10 mg/l) and one from the non-RA group, had a titre greater than 1/2 (1/8 in each case).

Table IV demonstrates the median APAs in RA patients on and off various categories of drugs. No drug appeared to influence APA titres.

DISCUSSION

This study, on a population independent from the one reported previously [1], has confirmed the association between elevated APAs and RA when the patient has a concomitant CRP > 10 mg/l. However, taking disease activity in a broader context, as is illustrated by the Stoke index, there was no correlation between this score and the APA, nor with a number of...
other single measures of disease activity. The longitudinal study demonstrated no correlation between changes in measures of disease activity and change in APA titre. This included change in CRP, which in contrast to our previous study narrowly missed being significantly associated with change in APA titre \((P = 0.06)\) [1]. The patient medication in the RA group did not appear to explain differences such as \textit{Clostridium perfringens} [5]. However, we were unable to confirm a study reported recently, which demonstrated a significantly higher isolation of \textit{Proteus} from the urine of RA patients compared with controls [11]. In the present study, \textit{Proteus} was only isolated in pure culture from three urines in the RA patients and one urine in the controls. In many of the other patients where \textit{Proteus} was isolated, it was presumably merely reflected contamination with organisms of faecal origin. There was also no association between faecal carriage of \textit{Proteus} and disease status. Furthermore, there is no evidence to suggest that the finding of \textit{Proteus} in urine or faeces influenced in any way the APA titre.

Following our observation that APA titres were higher in \(P\)\(_2\) blood group positive RA patients, and given that such individuals may be predisposed to asymptomatic bacteriuria [12], we were hopeful that isolation of \textit{Proteus} organisms or detection of APAs in the urine of RA patients would support the suggestion that the urinary tract acts as a sanctuary site for \textit{Proteus} in RA [2]. We were unable to demonstrate either phenomenon more frequently in the RA group. If our observation with regard to \(P\) blood group and APAs is confirmed, an alternative explanation for this observation has to be sought.

In conclusion, elevated APA titres are not associated with the activity of RA when measured as a composite, such as the Stoke index, or with individual measures of disease activity. The observation of elevation in RA patients with CRP>10 mg/l suggests that this antibody is acting as an acute phase protein, probably distinct from CRP in view of the lack of correlation between the two. It appears to be relatively specific to RA [2,3], and displays properties not shared with other antibacterial, anti-viral or autoantibodies [2,13]. However, the fact that it binds to \textit{Proteus} may be irrelevant, and the role of \textit{Proteus} in RA remains in doubt.

**Acknowledgements**

We thank our colleagues at the Freeman Hospital, Newcastle-upon-Tyne, Sunderland Royal Infirmary and Monkwearmouth Hospital, Sunderland, for allowing us access to their patients. We thank the nursing staff in these hospitals for their help in collecting the samples, and the patients for participating.

**References**


