Impaired delayed type cutaneous hypersensitivity in rheumatoid arthritis reversed by chrysotherapy

MALCOLM D SMITH, ANN SMITH, JOHN O'DONNELL, MICHAEL J AHERN, AND PETER J ROBERTS-THOMSON

From the Department of Clinical Immunology (Rheumatology Unit), Flinders Medical Centre, Bedford Park, South Australia 5042

SUMMARY A prospective 24 week study of 31 patients with active rheumatoid arthritis (18 women, 13 men) was undertaken to determine whether weekly intramuscular sodium aurothiomalate (gold) would influence delayed type cutaneous hypersensitivity (DTH) and other indices of cell mediated immunity. DTH to seven recall antigens was measured by Multitest on three occasions during the study. Twenty five patients completed the study. At entry 13 patients (12 female) were anergic, and no significant correlations were found between DTH and other clinical and immunological indices. Women showed a significantly greater depression of DTH than men. At week 24 only three of the patients were anergic with significant increase in mean DTH score being noted particularly to tuberculin, candida, and streptococcus. Improvement in DTH was observed in both gold responders and non-responders. In conclusion, patients with active rheumatoid arthritis show impairment of DTH, which is reversed by chrysotherapy. This effect is most apparent in women and appears to be relatively independent of the clinical response.

Key words: cell mediated immunity.

Many defects in various aspects of cell mediated immunity have been described in rheumatoid arthritis (RA).1 Delayed type cutaneous hypersensitivity (DTH) as defined by delayed skin reactions to common recall antigens is generally depressed in RA, though the mechanism is obscure.2-4 For example, Emery and colleagues found that 36% of their patients with RA were anergic to seven recall antigens and that this appeared to be independent of both nutritional factors and clinical and serological indices which reflected disease activity.2 More recently they have speculated that this impairment of DTH was due to defective production of interleukin 2 by T lymphocytes in RA.5 In the present study we assessed DTH and other clinical and immunological indices at regular intervals in 31 patients with active RA and determined the influence of chrysotherapy on these measurements over 24 weeks of treatment.

Patients and methods

PATIENTS AND CONTROLS
Thirty one patients with definite or classical RA (American Rheumatism Association criteria) (18 women, 13 men) with a mean age of 65·1 years (range 41–81) and a mean duration of disease of 9·4 years were entered into the study. All patients had active disease (presence of three or more inflamed joints and raised C reactive protein), and 28 were seropositive. All patients continued to receive non-steroidal anti-inflammatory drugs or supplemental analgesic drugs during the study (naproxen 11, diclofenac 5, sulindac 4, aspirin 4, piroxicam 3, ibuprofen 2, paracetamol 8); three continued to receive small constant doses of prednisolone and two continued with hydroxychloroquine. Two patients received three 1 g intravenous pulses of methyl prednisolone during week 1 of the study. All other drugs were unaltered during the study. Twenty five patients completed the 24 week course of weekly intramuscular sodium aurothiomalate (total dose 1130 g). Six patients withdrew:

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Correspondence to Dr Peter J Roberts-Thomson, Department of Clinical Immunology, Flinders Medical Centre, Bedford Park, South Australia 5042.
four from gold induced rashes at weeks 6, 16, 20, and 20; one moved interstate at week 12, and one died after operation at week 8.

DTH was also measured in 46 healthy subjects (21 men, 25 women) whose ages ranged from 15 to 64 years.

**Skin Testing**

DTH was measured using 'Multitest' (Institut Merieux, France) according to the manufacturer's instructions. This millipuncture applicator assesses cutaneous hypersensitivity to seven common recall antigens in a reproducible manner 48 hours after application. The diameter of induration for each antigen is recorded in millimetres and the final sum score calculated by addition of the mean diameters for each antigen. An anergic response is defined as an absence of dermal induration to all seven antigens.

**Clinical Assessment**

Patients were assessed clinically by the same observer at weeks 0, 4, 8, 16, and 24 of the study. Their articular index was determined (maximum possible score 78) together with a visual analogue score for pain (maximum possible score 30) and stiffness (maximum possible score 30) and physician global assessment (maximum possible score 30). The sum of these figures gave a clinical score (maximum possible 168). Gold responders were defined as those patients whose final clinical score at week 24 < 50% of the initial value, while non-responders were defined as those showing ≤20% change.

**Lymphocyte Measurement**

Circulating lymphocyte counts were determined at two week intervals, and lymphocyte subset analysis was performed at weeks 0, 4, 8, 16, and 24. Total white cell count was measured with a Coulter 5 plus 6 analyser and a 100 cell differential performed manually. Lymphocyte subset percentages were determined by flow cytometry (FACS IV) and monoclonal antibodies on peripheral blood mononuclear cell preparations as previously described with the exception that B cells were enumerated according to the presence of surface membrane immunoglobulin. Monocytes in the peripheral blood mononuclear cell preparations were gated out and multiple analyses with FMC33 (a monoclonal antibody reacting with the monocyte/macrophage lineage) showed that these cells constituted <5% of the analysed lymphocytes.

**Serological Studies**

Serological studies were performed at weeks 0, 4, 8, 16, and 24. Rheumatoid factor, C reactive protein, C1 esterase inhibitor, complement C3, C4, and C1q were measured by radial diffusion and intravenous agglutination respectively. Rheumatoid factor was determined using the latex agglutination test.
and IgG, IgA, and IgM were measured by rate nephelometry (Beckman's ICS), while circulating immune complexes were measured by the fluid phase Clq binding method of Zubler et al. Erythrocyte sedimentation rate was measured by the Westergren method.

**STATISTICAL ANALYSIS**

Multianalysis of variance was computed using the statistical package for the social sciences (SPSS) on the Flinders University prime computer. Pearson's correlations were used to compare variables at entry into this study. All data with the exception of DTH values were log transformed before analysis. Differences between groups were assessed using the Wilcoxon sum of ranks method. Significance was accepted if p<0.05.

**Results**

Twenty five of the 31 patients completed 24 weeks' treatment with intramuscular gold, and Table 1 lists the clinical, cellular, serological, and DTH scores obtained for these patients.

At entry 13 patients (12 female, one male) were anergic, but only three (all female) remained so at week 24. At entry there were no significant correlations between DTH scores and other clinical, cellular, and serological indices (as listed in Table 1), and this lack of significance persisted even when the anergic population was deleted from the analysis. Figures 1 and 2 show the mean (SD) DTH score per patient at weeks 0, 12, and 24. At week 24 the mean score for all patients and for women, but not men, was significantly greater than at week 0. Women, however, showed a significantly lower mean DTH score than men on each of the three occasions it was measured (Fig. 2, p<0.05) despite the fact that with the exception of the T4/T8 ratio other clinical, cellular, and serological indices were similar (Table 2). The mean (SD) DTH score obtained for the female control subjects was 15.3 (6.2) and for the men 20.4 (6.2). No healthy subject was found to be anergic. Figure 3 shows an analysis of DTH reactions to the seven separate antigens in female and male patients. Particularly noteworthy was the significant increase in DTH reactions to tuberculin.
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Table 2 Clinical, cellular, and serological features in men and women at entry into the study. Data are given as mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>Women (n=18)</th>
<th>Men (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.2 (9.9)</td>
<td>66.0 (9.4)</td>
</tr>
<tr>
<td>Clinical score</td>
<td>67.6 (26.1)</td>
<td>69.0 (25.9)</td>
</tr>
<tr>
<td>ESR† (mm/h)</td>
<td>56.6 (36.5)</td>
<td>59.7 (27.2)</td>
</tr>
<tr>
<td>CRP† (mg/l)</td>
<td>53.9 (37.0)</td>
<td>77.9 (43.4)</td>
</tr>
<tr>
<td>RF† (IU/ml)</td>
<td>806 (1250)</td>
<td>1180 (1360)</td>
</tr>
<tr>
<td>CIC† (U/ml)</td>
<td>17.2 (20.4)</td>
<td>29.1 (25.5)</td>
</tr>
<tr>
<td>IgG (g/l)</td>
<td>12.6 (5.2)</td>
<td>15.5 (3.88)</td>
</tr>
<tr>
<td>IgA (g/l)</td>
<td>3.64 (1.98)</td>
<td>3.93 (1.08)</td>
</tr>
<tr>
<td>IgM (g/l)</td>
<td>1.68 (0.65)</td>
<td>1.83 (0.84)</td>
</tr>
<tr>
<td>DTH (mm)</td>
<td>3.4 (5.8)</td>
<td>18.0 (14.8)*</td>
</tr>
<tr>
<td>T4/8 ratio</td>
<td>2.7 (2.5)</td>
<td>1.6 (0.58)**</td>
</tr>
</tbody>
</table>

*p=0.02; **p=0.06.
†ESR=erythrocyte sedimentation rate; CRP=C reactive protein; RF=rheumatoid factor; CIC=circulating immune complexes; DTH=delayed type cutaneous hypersensitivity.

Table 3 Mean delayed type cutaneous hypersensitivity score in gold responders and non-responders over 24 weeks of chrysotherapy.* Data are given as mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold responders (n=8)</td>
<td>12.1 (13.2)</td>
<td>20.8 (17.6)</td>
</tr>
<tr>
<td>Gold non-responders (n=8)</td>
<td>11.1 (17.8)</td>
<td>15.9 (17.9)</td>
</tr>
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</table>

*Multianalysis of variance for responders versus non-responders with respect to time (p=0.58).

(purified protein derivative) and candida (p<0.05, week 0 v week 24, Wilcoxon) in the women and streptococcus (p<0.05) in the men. It was also observed that the mean (SD) reaction size to tuberculin in rheumatoid men at week 0 (6.0 (6.3) mm) was greater than that in the healthy subjects (5.5 (2.3) mm), and this reaction size increased to 7.8 (6.3) mm at week 24. At the end of the study patients were divided into two groups, the gold responders and the non-responders, according to their clinical response. An increase in mean DTH reaction size was observed in both groups, with the gold responders showing a slightly greater but not statistically significant score than the non-responders (Table 3).

Discussion

The conclusions from this study are (a) there was a depression of DTH in patients with active RA, which was more marked in women; (b) there were no correlations between the DTH score at

Fig. 3 Mean (SD) delayed type cutaneous hypersensitivity (DTH) reaction size to seven individual recall antigens at weeks 0, 12, and 24 of chrysotherapy. The open histograms represent men and the filled histograms represent women. Multitest recall antigens were Tet=tetanus; Dipt=diphtheria; St=streptococcus; Tub=tuberculin; Gly=glycerine control; C=candida; Tri=trichophyton; Prot=proteus.
entry and other selected clinical, serological, and cellular indices; (c) there was a significant increase in the DTH score after 24 weeks of intramuscular gold; (d) this increase was observed in both the gold responders and the non-responders; (e) this increase was predominantly due to the return of DTH to tuberculin and candida in the women and to streptococcus in the men.

A number of observers have noted a depression of cutaneous DTH in RA. 2-5 In the current study 42% of the patients were anergic at entry, very similar to the 36% reported by Emery and colleagues, 2 who used the same Multitest applicator to assess DTH. In both studies none of the control subjects were anergic, but in our studies these controls were not matched for age with the patients and were generally younger. The mean DTH score obtained for our controls was very similar, however, to that reported by Emery et al, 2 whose control subjects were older (mean age 57 years) and were rheumatology patients with non-inflammatory joint diseases (mean [SD] DTH score of those patients was 18·0 (9·8) compared with 17·6 (6·2) for our younger control subjects). Hence we feel that despite the younger age of our control group the results indicate a marked depression of DTH to these seven common recall antigens in our rheumatoid patients. The pathogenesis of this depression is still obscure, however.

A variety of non-specific and specific factors are known which influence the DTH response. 8 These include: (a) gender; healthy women in general show a lower response of approximately 5 mm in the Multitest sum score than men; 9 (b) age; women, particularly, show diminished responses after the age of 65 years. 8 Our patients, in general, belonged to an older age group (mean age 65 years) and for the women this may have been one factor contributing to the diminished DTH responses, but the significant reversal noted after chrysotherapy suggests that this impairment was of an applied or secondary nature and that the age effect was of minor importance; (c) nutritional status; malnutrition suppresses DTH responses, but this does not appear to be relevant in RA; 2 (d) the presence of intercurrent medical conditions such as infections, renal impairment, anaemia, and cancer. Two of our patients (one male, one female) were subsequently found to have concurrent carcinomas and both were hypoorlgic or anergic at entry, one remaining so during the study. Immunosuppressive drugs are also potent inhibitors of DTH but were not relevant in the current study. It should be noted that aspirin does not influence cutaneous hypersensitivity, 9 and it can be deduced from the results of Emery et al, 2 whose control group contained many receiving non-steroidal anti-inflammatory drugs, that these drugs are also unlikely to influence cutaneous hypersensitivity responses greatly. Furthermore, repeated applications of Multitest do not influence the subsequent size of the delayed cutaneous responses and long term sensitisation does not appear to occur. 8 10 Of interest was the observation that the DTH response to tuberculin in our rheumatoid male population was at all times, despite their active disease, greater than that seen for the healthy controls. There are several possible explanations for this, but one recent publication suggests it may be related to the presence of the major histocompatibility complex DR4 phenotype 11 as such individuals are hyperreactive to tuberculin, and this phenotype would be strongly represented in our rheumatoid population. From our data the variable relating most obviously to the depressed DTH in RA was the female gender. Some investigators have speculated that the depressed DTH seen in RA may reflect a basic or intrinsic abnormality in the cellular immune arm in RA and have correlated it with depressed mononuclear cell proliferative responses in vitro, which are reversed by the addition of interleukin 2. 5 We have also observed significantly impaired peripheral blood mononuclear cell mitogenic responses in vitro in patients with RA compared with matched controls (unpublished data). In these studies no consistent correlation was observed between mitogenic responses and gender in the peripheral blood but, of interest to the present study, women showed a significantly impaired mitogenic response with OKT3 in the synovial fluid compartment. Further studies to assess gender differences in DTH and mitogenic responses will be required to clarify this issue.

It was also observed that our female patients showed a higher T4/T8 ratio than the men (p=0·06) at entry to the study, though age, disease activity, and other laboratory measurements were similar. Healthy women have significantly higher ratios than men, 12 but we are unaware of this difference being noted before in RA.

The depression in DTH was reversed after 24 weeks' treatment with intramuscular gold, though this effect appeared to be relatively independent of the clinical efficacy of this treatment. Why gold should have this effect is not known. Current thought suggests that intramuscular gold may have its most important effects on cells of the monocyte-macrophage lineage. It is possible that these cells and their cytokines could be defective in developing a normal DTH in RA and that gold corrects this disturbance. Further investigations along these lines are being pursued.

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