An investigation of the action of disease modifying antirheumatic drugs on the rheumatoid synovial membrane: reduction in T lymphocyte subpopulations and HLA-DP and DQ antigen expression after gold or penicillamine therapy

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SUMMARY Synovial needle biopsy specimens from the knee joints of seven patients with rheumatoid arthritis (RA) were examined immunohistochemically before and after six months’ treatment with either gold or penicillamine (disease modifying drugs, DMDs). There were significant reductions in the numbers of infiltrating T lymphocytes and a disproportionate fall in the numbers of lymphocytes of the helper/inducer subset when compared with those of the suppressor/cytotoxic subset. This resulted in a fall in the ratio of helper/inducer to suppressor/cytotoxic cells. The immunohistological changes correlated with improvements in erythrocyte sedimentation rate (ESR), serum immunoglobulins, visual analogue pain assessment, grip strength, and Ritchie articular index. A second group of nine patients with RA, already well established on DMD therapy, did not show similar changes after the six month period. The HLA class II antigens DR, DQ, and DP were widely expressed on lymphocytes, macrophages, and synovial lining cells of a group of patients with RA who had never received disease modifying drug therapy. After treatment there was a significant reduction in the expression of HLA-DP and DQ antigens.

Key words: rheumatoid arthritis, HLA class II antigens.

Rheumatoid arthritis (RA) is a chronic inflammatory disorder with a destructive, erosive effect on hyaline cartilage in peripheral joints. The evidence points to an excessive and prolonged stimulation of the immune system, with the synovial membrane (SM) as a major target organ. Indeed the SM can be likened to active lymphoid tissue with infiltrates of T and B lymphocytes and macrophages.1 The presumed stimulating agent, i.e., antigen, has long eluded identification; viral particles have sometimes been found within affected joints2,3 and a transient or prolonged arthritis may follow viral illnesses.3

Treatment with non-steroidal anti-inflammatory drugs (NSAIDs) alleviates symptoms and suppresses humoral inflammatory mediators but probably does not influence the long term outcome of the erosive joint disease. Drugs such as gold and penicillamine are believed to have a more fundamental effect on the disease process and have thus been called ‘disease modifying’. Although both these drugs improve clinical and laboratory parameters of disease activity in some patients with RA during the first six months of treatment,4 their effect on retardation of the disease process in the much longer term and their mechanism of action remain in doubt. When the activity of the disease process in RA is determined, reliance is usually placed on clinical measurements such as the duration of early morning stiffness, visual analogue pain score,5 grip strength, or Ritchie articular index6 and laboratory indices such as erythrocyte sedimentation rate, haemoglobin, C reactive protein, and other acute

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phase reactants, which may all signify an inflammatory reaction but may not accurately reflect alterations in the diseased synovium. We have, therefore, studied needle biopsy specimens of the SM from patients with active RA who had not previously received DMD therapy in an attempt to show the effect of these drugs on the rheumatoid SM T lymphocyte subpopulations and the HLA class II antigen expression during the first six months of treatment. The results have been correlated with changes in clinical and laboratory parameters of disease activity. We have compared these findings with those for a second group of patients with RA who were already well established on DMDs, but who still had evidence of active disease.

Patients and methods

Patients entering the study were selected from those attending the rheumatology unit of Southampton General Hospital. All were seropositive for rheumatoid factor and fulfilled the American Rheumatism Association criteria for classical or definite RA. Two groups were defined: group 1 patients were judged to have active RA with synovitis of at least one knee joint and were about to commence either sodium aurothiomalate (Myocrisin) by intramuscular injection or d-penicillamine (Distamine) orally, in conventional doses. These patients had not previously received either of these drugs and the choice of drug was left to the individual clinician’s preference. Group 2 patients had active synovitis of at least one knee joint but were already established on a DMD, which was continued unaltered throughout the assessment period.

On entry into the study a full clinical examination was performed which included body weight, duration of early morning stiffness (EMS), the patient’s assessment of overall joint pain in the previous week using a 100 mm visual analogue pain scale (VAPS), grip strength (GS), and a modified Ritchie articular index (RAI). Laboratory investigations were full blood count, erythrocyte sedimentation rate (ESR) (Westergren), serum immunoglobulins (Ig) G, A, and M, C reactive protein (CRP), β2 microglobulin (β2M), immune complexes by polyethylene glycol precipitation and sheep cell agglutination titre (SCAT). Whenever possible synovial fluid was obtained for the measurement of HLA class II soluble material.

Patients were reviewed clinically at 12 weeks and after 24 weeks when all laboratory tests were also repeated.

**IMMUNOHISTOCHEMICAL EXAMINATION OF SYNOVIAL MEMBRANE SECTIONS**

A synovial biopsy specimen was taken under local anaesthesia from the suprapatellar pouch of an affected knee joint with a Cope pleural biopsy needle. Whenever possible several pieces of tissue were obtained from a small area in the midline of the pouch. Specimens were wrapped in aluminium foil and after labelling, immediately snap frozen in liquid nitrogen and stored over the same for subsequent analysis of the cell populations present. The procedure was repeated after 24 weeks with a specimen taken from the same area in the same knee joint. This enabled comparison with the first biopsy specimen and minimised sampling error that might occur due to variation of the intensity of the SM inflammatory process within different compartments of the knee joint. Each biopsy specimen was examined under light microscopy by an independent consultant histopathologist (Dr P J Gallagher) to confirm its SM origin.

An indirect immunoperoxidase method modified from Gregg et al was used to examine the synovial cell populations. Specimens were retrieved from storage, briefly thawed, then embedded in OCT medium (Tissue Tek Ltd) and rapidly refrozen to −20°C in a cryostat. Serial, 6 μm sections were laid on glass slides and air dried before overnight storage on silica gel. The following day the slides were air dried for five minutes, fixed for 20 minutes in dry acetone at 4°C, and then washed in TRIS (trometamol)/HCl buffered saline pH 7.6 (TBS). Subsequent procedures were carried out at room temperature in a humidified chamber. The serial sections were incubated for 30 minutes in 30 μl of a murine monoclonal antibody (MoAb). These antibodies (specificities in parentheses) included: UCHT1 (anti-T lymphocytes), HB2 (anti-T helper inducer lymphocytes), OKT8 (anti-T suppressor/cytotoxic lymphocytes), Leu 8 (regulatory population of T lymphocytes), RFB4 (anti-B lymphocytes). The following MoAbs were used to define HLA class II antigens: FMC4 directed towards HLA-DR, TU22 directed towards HLA-DQ, B7/21 directed towards HLA-DR, and DA6/23 directed towards HLA-DR, DQ, and DP. These antibodies had previously been titrated on a synovectomy SM specimen, for optimal staining. A MoAb directed against a non-human epitope was used as a negative control.

After two, three minute washes in TBS 30 μl of peroxidase conjugated rabbit antimouse Ig (Dakos Ltd), at a dilution of 1:80 and containing AB serum, was applied to the slides for 30 minutes. The slides were then washed twice in TBS, incubated in a solution of 3-amino-9-ethylcarbazole and 1% hydrogen peroxide for 10 minutes, washed in tap water, counterstained with Mayer’s haemalum, and finally, mounted in glycerol jelly under glass coverslips.
Disease modifying antirheumatic drugs

QUANTIFICATION OF SYNOVIAL MEMBRANE T LYMPHOCYTE SUBSETS

The SM sections were examined for the total number of T lymphocytes and the subsets bearing the helper/inducer (T4+) and suppressor/cytotoxic (T8+) surface cell markers.

SM sections were also stained with the MoAb Leu 8 in an attempt to demonstrate the cell populations defined by this antibody, which has been shown in peripheral blood to delineate a regulatory population of T cells.12

A Leitz Wetzlar Dialux microscope with an eyepiece mounted graticule was used to measure the number of lymphocytes present on each section, so that at 500 times magnification, under oil immersion, the area visible within one graticule grid field (GF) was 162 μm². A systematic count across and down each section, and covering the whole surface of the section, provided a mean cell count per unit area for each T lymphocyte subset for each slide. Areas showing a fibrous exudate were not counted. As there was variation in biopsy specimen sizes a fixed interval between each GF counted was predetermined for each slide to limit the GFs counted to a practical number.

All sections were read by MTW without knowledge of patient identity or clinical grouping. In order to identify any variation in histopathology within the suprapatellar pouch, midline region a second sample of tissue was taken in some patients from an adjacent area of synovium within 2–3 cm of the first biopsy specimen and stained in parallel. Both biopsy specimens were then assessed for the degree of correlation of T lymphocyte infiltration on a 0–6 visual scale under low power microscopy.

QUANTIFICATION OF SYNOVIAL MEMBRANE HLA CLASS II ANTIGEN EXPRESSION

First biopsy SM sections from group 1 patients and two other patients with RA receiving a NSAID only were examined for the presence of the HLA class II antigens DR, DQ, and DP. These were compared with SM biopsy sections from patients who had received at least six months' DMD therapy and included some patients from group 2. To measure the amount of class II antigen present an assessment over the whole section was made under low power microscopy using a 0 to 5 visual scale. A score of 0 represented absent staining and 5 the most intense staining. Each slide was read independently by MTW, JLS, and KM. To test interobserver variation in allocating a score to a given slide by this method 58 random SM sections were stained for the presence of HLA-DR and read independently by each observer. The correlation was assessed by the Mann-Whitney U test. Other statistical analyses were performed using the paired t test (Tables 1 and 2) and the standard Student's t test (Table 3) to compare the results before and after the six months' observation period.

Results

Sixteen patients completed the study. Group 1 (n=7, five male, two female) patients had a mean age of 53-5 years (range 35–70) and a mean disease duration of 3-1 years (range 0-25–7-5). At the beginning of the study four group 1 patients received penicillamine and three sodium aurothiomalate. One group 1 patient developed a rash after receiving gold for three months and was changed to azathioprine. There were no other drug side effects. All patients reported an improvement in their overall condition, and in some this was marked. Group 2 (n=9, four male, five female) patients had a mean age of 64 years (range 45–76), a mean disease duration of 11-1 years (range 2-4–26), and had been receiving DMD therapy (usually gold or penicillamine) for a mean of 10-3 years (range 2-24) before entering the study.

CHANGES IN CLINICAL INDICES OF DISEASE ACTIVITY (Table 1)

There were no statistically significant differences in the mean values of the clinical indices of disease

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Changes in clinical indices of disease activity</th>
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<tbody>
<tr>
<td></td>
<td>Group 1 (n=7)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66 (51–101)</td>
</tr>
<tr>
<td>EMS (min)</td>
<td>89-3 (5–210)</td>
</tr>
<tr>
<td>VAPS (0–100 mm)</td>
<td>69 (41–88)</td>
</tr>
<tr>
<td>GS (mmHg)</td>
<td>105 (45–177)</td>
</tr>
</tbody>
</table>

Mean values (range) are given.

There were no statistically significant differences between groups 1 and 2 at the beginning of the study (p>0-05, Mann-Whitney U test).

*p<0-05; **p<0-01 (paired t test). There were no other significant changes.
Fig. 1  (a) Reduction in rheumatoid SM T lymphocytes after six months' DMD therapy. (b) Reduction in rheumatoid SM T helper/inducer lymphocytes after six months' DMD therapy. (c) Reduction in rheumatoid SM T suppressor/cytotoxic lymphocytes after six months' DMD therapy. (d) Reduction in rheumatoid SM T helper/inducer:suppressor/cytotoxic lymphocytes ratio after six months' DMD therapy.
activity measured at the beginning of the study in either group (p > 0.05, Mann-Whitney U test).

Significant improvements occurred over the six month period in group 1 but not group 2 patients in body weight, VAPS, GS, and RAI. Although EMS diminished in both groups, this was not significant in group 1 but was significant in group 2, which was against the trend of the other clinical measurements in that group.

CHANGES IN LABORATORY INDICES OF DISEASE ACTIVITY (Table 2)

The mean values of all indices measured were similar at the beginning of the study with no statistically significant differences between the groups (p > 0.05, Mann-Whitney U test). Significant improvements occurred in group 1 but not group 2 patients in ESR (mean 92 to 56 mm/1st h, p < 0.01) and serum IgG (mean 18.7 to 14.8 g/l, p < 0.05) and IgM (mean 1.81 to 1.27 g/l, p < 0.02) concentrations. Although the level of CRP almost halved in group 1 (mean 0.119 to 0.069 g/l), the decrease did not reach statistical significance. β2M levels did not change significantly in either group. Immune complexes were found in 3/7 group 1 patients before treatment and 1/7 patients after the six months’ observation period. The corresponding figures for group 2 were 3/9 and 1/9. The SCAT tended to fall in group 1.

CHANGES IN SYNOVIAL MEMBRANE T CELL POPULATIONS (Figs 1a–d)

A mean of 69 GFs was counted for each biopsy section (range 24–139). All group 1 patients had infiltrates of T lymphocytes in the pretreatment biopsy specimens (mean 22.6 cells/GF, range 11–40). After six months’ DMD therapy all group 1 patients showed significant falls in the total number of T cells present (mean 7.4 cells/GF, range 1.5–20, p < 0.05). Similarly, the mean number of T4+ cells per unit area of GF fell from 13.2 to 2.9, p < 0.02, and the T8+ cells fell from 11.8 to 4.6, p < 0.02 in the group 1 patients. The ratio of T4/T8 cells (immuno-regulatory ratio20) fell significantly in group 1 owing to the disproportionate fall in the T4+ cell population relative to the fall in the T8+ cell population. Patients in group 2 did not show significant reduction in any of the T cell populations studied, nor in the T4/T8 ratio. For both groups the sum of the T4+ and T8+ lymphocytes per unit area of GF approximated that of the total T lymphocytes.

ASSESSMENT OF HISTOPATHOLOGICAL VARIATION IN BIOPSY SPECIMENS

Thirteen pairs of biopsy specimens taken simultaneously were successfully stained for total T, T helper/inducer, and T suppressor/cytotoxic lymphocytes (group 1, four pretreatment, five post-treatment patients, group 2, two patients, and also two patients receiving a NSAID only). There was no significant difference in the intensity of infiltration of the total number of T lymphocytes or either subset (p > 0.05, paired t test and Mann-Whitney U test) when compared on the 0–6 visual scale described above. Fig. 2 shows the typical appearance of a SM section stained for T lymphocytes.

STAINING OF SM SECTIONS WITH ANTI LEU 8

This antibody stained lymphoid cells but also much intercellular material that was difficult to define. Vascular endothelium was strongly positive.

HLA CLASS II ANTIGEN EXPRESSION

(Figs 3–5)

HLA class II antigens were widely distributed within the synovial membrane. HLA-DR was always the most widely expressed and was found on macrophages, synovial lining cells, B and T cells. HLA-DQ and DP were also found, generally less widespread than DR and were expressed on macrophages, lymphocytes, and synovial lining cells in variable amounts. The endothelium of an occasional small blood vessel had weak expression of HLA-DR but not DQ or DP. There was good correlation between observers in assessing the 58 HLA class II stained SM sections (p > 0.05, Mann-Whitney U test). The changes in HLA class II expression after at least six months’ DMD therapy are shown in Table 3. Although HLA-DR expression was reduced, it did not reach statistical significance, but significant reductions in HLA-DP and DQ expression were found. Five patients receiving a NSAID, examined for changes in HLA-DR expression only, showed no change after a six month period.

HLA-DQ was absent on some cells stained with

<p>| Table 2 Changes in laboratory indices of disease activity |
|----------------------------------|----------------------------------|</p>
<table>
<thead>
<tr>
<th><strong>Group 1 (n=7)</strong></th>
<th><strong>Group 2 (n=9)</strong></th>
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</thead>
<tbody>
<tr>
<td><strong>Hb (g/l)</strong></td>
<td><strong>Hb (g/l)</strong></td>
</tr>
<tr>
<td>10.7</td>
<td>11.7</td>
</tr>
<tr>
<td>10.3</td>
<td>9.8</td>
</tr>
<tr>
<td><strong>ESR (mm/1st h)</strong></td>
<td><strong>ESR (mm/1st h)</strong></td>
</tr>
<tr>
<td>92</td>
<td>56**</td>
</tr>
<tr>
<td>83</td>
<td>79</td>
</tr>
<tr>
<td><strong>Ig (g/l)</strong></td>
<td><strong>Ig (g/l)</strong></td>
</tr>
<tr>
<td>18.7</td>
<td>14.8*</td>
</tr>
<tr>
<td>14.2</td>
<td>15.5</td>
</tr>
<tr>
<td><strong>A</strong></td>
<td><strong>A</strong></td>
</tr>
<tr>
<td>4.11</td>
<td>2.63</td>
</tr>
<tr>
<td>3.3</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>M</strong></td>
<td><strong>M</strong></td>
</tr>
<tr>
<td>1.81</td>
<td>1.27**</td>
</tr>
<tr>
<td>1.4</td>
<td>1.4</td>
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<tr>
<td><strong>CRP (g/l)</strong></td>
<td><strong>CRP (g/l)</strong></td>
</tr>
<tr>
<td>0.119</td>
<td>0.069</td>
</tr>
<tr>
<td>0.098</td>
<td>0.076</td>
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<tr>
<td><strong>β2M (g/l)</strong></td>
<td><strong>β2M (g/l)</strong></td>
</tr>
<tr>
<td>5.34</td>
<td>4.96</td>
</tr>
<tr>
<td>5.58</td>
<td>6.06</td>
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</tbody>
</table>

Mean values are given.

There were no statistically significant differences between groups 1 and 2 at the beginning of the study (p > 0.05, Mann-Whitney U test).

*p < 0.05; **p < 0.02; ***p < 0.01 (paired t test). There were no other significant changes.
Fig. 2  Rheumatoid SM stained for T lymphocytes with the MoAb UCHT1. There is a large aggregate of cells around one blood vessel (X), while other vessels (V) are surrounded by few T lymphocytes. (Immunoperoxidase).

Fig. 3  Section of a SM biopsy specimen from a patient with active RA treated with a NSAID only, stained for HLA-DR. Widespread expression of HLA-DR on lymphoid cells, macrophages, and synovial lining cells. (Immunoperoxidase).

Fig. 4  Section of a SM biopsy specimen (adjacent to that in Fig. 3) from a patient with active RA treated with a NSAID only, stained for HLA-DQ. HLA-DQ expression is mainly confined to the aggregate of lymphoid cells (\), most of which are T4+ and very few T8+. This area is strongly positive for HLA-DR (Fig. 3). Some synovial lining cells are positive. (Immunoperoxidase).

RFB4 and was also absent on T8+ cells in one area of a section from a representative patient with active disease receiving a NSAID only.

Discussion

Although RA is a heterogeneous disease in the sense that there is a wide variation in the severity and destructive potential of the synovial inflammation, there is qualitative similarity in respect of the underlying synovial histopathology. The characteristic features are dense, mixed leucocyte infiltrations with a predominance of mononuclear cells, especially lymphocytes and plasma cells. There is increased vascularity, synovial hypertrophy, and fibrinous exudation into the synovial space. Much
evidence points to an immunological destructive process within the host tissues, including synovial membrane, though the underlying stimulus to the reaction is unknown.

This is the first longitudinal study of which we are aware that has attempted to measure quantitatively and qualitatively the changes due to the action of DMDs on the rheumatoid SM lymphocyte sub-populations and HLA class II antigen expression. Previous reports on the cellular composition of the rheumatoid SM have relied upon tissue obtained at surgical synovectomy (usually the hip joint), and as a consequence disease has usually been long standing. By examining rheumatoid SM at an early stage in the disease process, and before treatment with a DMD, it is hoped that any influence of secondary degenerative change upon the tissues can be avoided. By comparing these findings with those for a group of patients well established on second line therapy, any regression or cumulation of effect might become apparent.

The patients in group 1 all had severe disease when expressed in terms of ESR, duration of EMS, VAPS, GS, and RAI. All patients from both groups were seropositive, and some patients from both groups had circulating immune complexes. All group 1 patients improved after six months' treatment with DMD therapy as measured by weight gain, fall in ESR, IgG, and IgM, reduction in VAPS, RAI, and improvement in GS. Group 2 patients showed no significant changes in clinical or laboratory indices, apart from an improvement in EMS, which is a subjective test. We chose patients with active synovitis of a knee joint for group 2 as it seemed preferable to select patients in whom a synovial biopsy could be most easily performed by the blind, needle method. These group 2 patients can be regarded as having only partially responded to DMD therapy. Thus we have studied two distinct groups of patients with active RA: group 1 in whom we would anticipate improvement after six months' DMD therapy and group 2 who were well established on DMD therapy and would not necessarily be expected to show further improvement during the course of the study.

Previous studies using monoclonal antibodies have shown characteristic distributions of lympho-

![Fig. 5](image_url)

**Fig. 5** Section of a SM biopsy specimen (adjacent to that in Fig. 4) from a patient with active RA treated with a NSAID only, stained for HLA-DP. HLA-DP expression is mainly confined to the lymphoid cells positive for HLA-DR and DQ. (Immnooperoxidase).

<table>
<thead>
<tr>
<th>MoAb</th>
<th>Pre-DMD</th>
<th>Post-DMD</th>
<th>NSAID only (n=5)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st biopsy</td>
<td>2nd biopsy</td>
</tr>
<tr>
<td>FMC4 (DR)</td>
<td>2-8†</td>
<td>2-1† p NS</td>
<td>2</td>
</tr>
<tr>
<td>DA6231 (DR,DQ,DP)</td>
<td>3-6*</td>
<td>2-8† p NS</td>
<td>1-9 p NS</td>
</tr>
<tr>
<td>TGU22 (DQ)</td>
<td>2-9*</td>
<td>1-3‡ p&lt;0-01</td>
<td>—</td>
</tr>
<tr>
<td>B721 (DP)</td>
<td>2-0*</td>
<td>1-1‡ p&lt;0-01</td>
<td>—</td>
</tr>
</tbody>
</table>

Mean values on 0-5 scale.

*+=7; ‡=9; †=10. p Values determined by Student's t test.

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*Disease modifying antirheumatic drugs* 13

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Table 3  HLA class II antigen expression in rheumatoid synovial membrane before and after six months' treatment with a DMD
cyte subsets within the rheumatoid synovium. Aggregates of T4+ cells interspersed with a few T8+ cells can be found surrounding blood vessels and also as distinct lymphoid follicles. 21 These T4+ cells may act cooperatively with antigen presenting cells of the macrophage lineage which simultaneously express DR antigens to induce B cells to secrete immunoglobulin, resulting in localised complement fixation and subsequent tissue damage. T8+ cells have also been found in close association with B lymphocytes, and it is believed that these cells act as functional suppressors on the T4+ subset. This simple model has allowed us to examine the in situ characteristics of cells with previously defined functional properties.

Group I patients had significant reductions in the absolute numbers of infiltrating T lymphocytes and both T4+ and T8+ subsets (to levels similar to those in group 2) after six months' treatment with conventional doses of gold or penicillamine (Figs 1a–c). This fall was most marked for the T4+ subset, which consequently resulted in a fall in the T4/T8 ratio to a level similar to that for group 2 patients, who did not show significant falls in either T cell subset (Fig. 1d).

The ratio of T4/T8 cells is often quoted to reflect the proportions of these regulatory T cells present and is usually high if calculated from perivascular areas, where there is a predominance of T4+ cells. 1 21 This would ignore potentially functional T8+ cells found more peripherally. We found a non-random distribution of T cell subsets in that the T8+ population was preferentially located in the areas between blood vessels of the SM, but frequently there were large numbers of T8+ cells mixed with T4+ cells surrounding blood vessels. Thus in measuring the lymphocyte subsets present, we have considered all areas of the SM, including the areas between blood vessels. The T4/T8 ratio in our patients was therefore lower than in previous reports. This may reflect more accurately the balance between these regulatory subsets and eliminates any observer bias in the counting process.

We found good consistency in the total number of T lymphocytes and T subsets infiltrating the biopsy specimens taken from adjacent areas of the suprapatellar pouch SM by this blind, needle biopsy method. Rooney et al found on arthroscopic SM biopsies that in individual patients with RA there is homogeneity of the histological changes within different areas of the knee joint, and we are also addressing this question. 22 We advocate the use of the Cope biopsy needle rather than the smaller Parker Pearson needle as the instrument is easier to handle and yields larger biopsy specimens.

The expression of the HLA class II antigens DQ and DP was reduced after DMD therapy. Some of the positively stained class II material appeared to be lying free within the intercellular spaces and may have been shed from cells. An enzyme linked immunosorbent assay was developed by members of our group to detect these soluble class II products in the RA synovial fluid, and this has been reported elsewhere. 23

The reduction of HLA class II expression could suggest that there are fewer macrophages present, and it has been shown that gold salts can affect the phagocytic properties of macrophages. 24 It may be that the reduction in the numbers of infiltrating T lymphocytes after DMD therapy reflects diminished stimulation by antigen presenting cells. Penicillamine, however, has been shown to suppress the function of T helper lymphocytes in vitro, 25 and thus it is difficult to be certain in what manner these drugs affect the inflammatory response in the diseased rheumatoid synovium. To answer this will require further analysis of the biopsy sections with double labelling methods to detect the presence of T cell activation antigens.

In one patient with active RA, chosen for more detailed examination, in whom the gross lymphocytic infiltrates were not dissimilar from those of others in the study, there was an interesting distribution of HLA class II antigen within adjacent lymphoid aggregates. In one area there was heavy expression of HLA-DR, much HLA-DQ, and many T4+, and few T8+ and B lymphocytes (Figs 3–5). This suggests that the T4+ cells were activated, as it is recognised that both HLA-DR and DQ are associated with T cell activation. 26 In an adjacent lymphoid aggregate there were few T4+ cells, but many T8+ and B cells. Here there was little expression of HLA-DR, which was confined to cells with the morphological appearance of macrophages, and no expression of HLA-DQ. This lack of expression of HLA-DQ on T8+ cells is interesting in the light of the evidence from Navarrete et al that HLA-DQ is associated with activated T8+ suppressor cells. 27 This might suggest that the T8+ cells in this area were not activated suppressor T cells and therefore unable to modulate the inflammatory response. Others too have noted a complete lack of suppressor activity in eluted RA SM lymphocytes. 28 Thus there seem to be diverse states of immunological activity within even adjacent areas of the rheumatoid SM, and it will be interesting to see in further studies if these preliminary findings are consistent.

Recent evidence has been presented to show that the definition of OKT4+ lymphocytes as helper cells is no longer absolute. MoAbs have been produced that define subsets of these cells as either suppressor/inducer cells 12 or as directly suppressing...
B cell Ig production.\(^{29}\) In view of this we have attempted to use the MoAb Leu 8, which identifies T4+ suppressor/inducer cells, to define more accurately the potential function of the T4+ lymphocytes present within the rheumatoid SM. The results with this MoAb were disappointing as it frequently stained non-specifically many synovial cells and vascular endothelium. We are currently using the MoAb WR16,\(^{29}\) which is lymphocyte specific, to define more rigorously the subsets of T4+ lymphocytes within the SM.

One of the principal aims of drug therapy in RA has been the suppression of the disease process, though the drugs which achieve this are limited in use by their toxicity. Nevertheless, well publicised controlled trials with gold, D-penicillamine, and other slow acting drugs have shown significant improvement in both clinical and laboratory parameters of disease activity which became apparent within six months.\(^3\) Trials have not continued for long enough, however, to assess accurately the effect of these drugs over more prolonged periods of several years.

We have attempted to measure the effect of DMD therapy in RA over a six month period by analysing changes in both cellular components of the inflamed synovium and expression of HLA class II antigens. Our preliminary findings indicate that these drugs suppress the inflammatory reaction in the SM during the first six months of treatment, and this is in accordance with clinical observations.

Previous attempts to correlate synovial histology with the severity of disease have usually been unsuccessful.\(^{30}\) We have shown that in 13 paired biopsy specimens the nature of the T cell infiltrate did not significantly vary over the whole SM tissue section. Preliminary evidence, however, suggests that there may be variation of HLA class II antigen expression in adjacent lymphoid aggregates. In a follow up study we are continuing to address the question of whether there is variation in the intensity of the inflammatory infiltrate within different regions of the RA synovium, but our results from the present study suggest that blind, needle synovial biopsy specimens taken from the same area, in a longitudinal study, may be a useful means of assessing the tissue effects of established and new therapies for rheumatoid arthritis.

We would like to thank Dr P J Gallagher for his help in assessing the synovial histopathology in this study. This work was carried out while MTW was the Arthritis and Rheumatism Council Copeman research fellow, with supplementary financial support from Dista Pharmaceuticals Ltd.

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An investigation of the action of disease modifying antirheumatic drugs on the rheumatoid synovial membrane: reduction in T lymphocyte subpopulations and HLA-DP and DQ antigen expression after gold or penicillamine therapy.

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