Cladribine in the treatment of systemic lupus erythematous nephritis

Systemic lupus erythematosus (SLE) nephritis often requires treatment with cyclophosphamide, which carries the risk of major side effects including infection, ovarian failure and bladder malignancy. Therapeutic strategies that would specifically target lymphocytes are appealing. Following the first report of the use of the purine nucleoside analogue cladribine (2-chloro-2-deoxyadenosine), a selective lymphocyte depleting agent, in the treatment of lupus nephritis, we report our experience in two patients with severe renal involvement.

CASE 1
A 32 year old woman was diagnosed with SLE at age 28, with polyarthritides, photosensitive rash, subcutaneous nodules, fatigue and lymphopenia. ANA, anti-dsDNA, anti-Sm and anti-RNP antibodies were present. Various immunosuppressants and corticosteroids failed to maintain a sustained remission. Two and a half years after presentation, she developed haematuria and proteinuria and renal biopsy revealed WHO Class III lupus nephritis. Treatment with pulsed intravenous cyclophosphamide and methyprednisolone had to be stopped after four months and a total cyclophosphamide dose of 9 g because of an anaphylactic reaction during an infusion. Despite azathioprine, 150 mg/day, and prednisone, up to 20 mg/day, she developed severe hypertension (210/120 mmHg) and biopsy confirmed lymphocytic cutaneous vasculitis. Cladribine (0.05 mg/kg/day for seven days as continuous intravenous infusion) and prednisolone 40 mg/day proved ineffective as creatinine rose from 149 to 243 µmol/l in two months. She also developed a perineal herpes simplex infection but drug was otherwise well tolerated. Pulse intravenous cyclophosphamide and methyprednisolone were subsequently reintroduced and creatinine has again fallen to 118 µmol/l.

Table 1 shows the results of investigations before and after cladribine infusions for both cases.

In the initial study by Davis et al., three of seven patients treated with continuous cladribine infusion for a week responded completely and renal function did not deteriorate in any of the seven patients. Our limited experience suggests that cladribine may be effective in other manifestations of SLE (that is, cutaneous vasculitis), but it does not seem to have a consistent effect in severe nephritis. Good tolerability of the drug was confirmed and although herpes simplex infections occurred in both patients the role of corticosteroids cannot be ignored.

Further studies are required to establish the position of cladribine in the treatment of SLE especially in the presence of other lymphocyte depleting agents such as mycophenolate mofetil, which is reported to be effective in lupus nephritis.11 Even in cases refractory to cyclophosphamide.12

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Table 1 Results of investigations before and after cladribine infusions

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>12.25 g/24 h</td>
<td>4.2 g/24 h</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>190 µmol/l</td>
<td>120 µmol/l</td>
</tr>
<tr>
<td>Anti-ds DNA</td>
<td>132 IU/ml</td>
<td>58 IU/ml</td>
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<tr>
<td>C3</td>
<td>0.51 g/l</td>
<td>0.72 g/l</td>
</tr>
<tr>
<td>C4</td>
<td>0.13 g/l</td>
<td>0.15 g/l</td>
</tr>
<tr>
<td>C3d</td>
<td>22 units/ml</td>
<td>10 units/ml</td>
</tr>
<tr>
<td>Urine analysis</td>
<td>red cells, hyaluronic, cellular casts</td>
<td>red cells, hyaluronic, cellular casts</td>
</tr>
</tbody>
</table>

Reference ranges: serum creatinine 50–100 µmol/l, anti-dsDNA: 50–300 IU/ml positive, >300 IU/ml strongly positive, C3: 0.63–1.19 g/l, C4: 0.11–0.43 g/l, C3d: up to 12 units/ml.

Leg bone pain syndrome in a kidney transplant patient treated with tacrolimus (FK506)

Patients with chronic renal failure often develop musculoskeletal problems such as renal osteodystrophy and amyloid arthropathy,1 and in successful renal transplantation other complications may ensue, particularly avascular necrosis.2 Since the availability of immunosuppressive agents for rejection, there has been a decrease in musculoskeletal problems, however, new complications have been described such as a symmetrical bone pain syndrome and reflex sympathetic dystrophy syndrome (RSDS), some of them related to cyclosporin.3

Tacrolimus is a novel macrolide with potent immunosuppressive effects and with a very similar mechanism of action to cyclosporine A—that is, calcineurin phosphatase inhibition.4 We report on a patient treated with tacrolimus, who developed a leg bone pain syndrome, two months after kidney transplantation.

The patient was a 50 year old woman with severe hypertension, treated with atenolol (100 mg/day), verapamil (240 mg/day) and clonidine (0.150 mg/day). She developed chronic renal failure and was treated with peritoneal dialysis in 1995. In 1997 she underwent a kidney transplant from a cadaver and immunosuppressive treatment with tacrolimus (4 mg/day) and prednisone (15 mg/day) was started. Two months after transplantation she reported progressive bilateral symmetric pain in the knees. Because of pain and difficulty in walking she was readmitted to our unit. At this time, the patient was receiving tacrolimus (4 mg/day) and prednisone (5 mg/day). Clinical examination revealed pain on movement and tenderness over the bone and joint line, without swelling.

Figure 1 Bone scintigraphy, showing increased uptake in both knees.

or increased temperature. She had no signs of autonomic vasomotor disturbances and arteriolar mobility was normal. Examination of the remaining peripheral and axial joints was normal.

Blood tests showed creatinine levels of 1.3 mg/dl, calcium of 10.1 mg/dl, phosphate of 3.5 mg/dl and urate of 7.2 mg/dl. Other laboratory findings were normal. Patchy osteoporosis in the knees was seen radiographically.

Bone scintigraphy showed intense uptake in both the osseous and vascular phases in the knees (fig 1). Calcitonin treatment was begun (three monthly cycles of 10 intramuscular units/day during 20 days) without clinical improvement. Because of the high serum concentrations of tacrolimus (15 μg/ml) and the ineffective calcitonin treatment, tacrolimus was reduced to 2 mg/day. Nine months after transplantation, she was free of symptoms and radiographs and tacrolimus concentration (5.1 μg/ml) were normal. Changes in plasma tacrolimus concentrations subsequent to the resolution of symptoms did not occur and the patient continued asymptomatic.

We describe a complication in a patient treated with tacrolimus after kidney transplantation that is similar to that described by other authors in transplanted patients treated with cyclosporin. Although the radiographic and bone scintigraphy findings suggested RSDS, the symptoms of this patient were not the classic features of this entity. The efficacy of corticosteroids in the treatment of uncompli-
cated RSDS has been demonstrated,4 so it is possible that corticosteroids might have a protective role against a full RSDS development, as she was treated with high doses of prednisone after the renal transplantation.

The early onset of symptoms after the administration of the drug and the clinical improvement after the reduction of the immunosuppressive dose, are features that support a possible relation between tacrolimus and the leg bone pain syndrome. The patient had high plasma tacrolimus concentrations at the onset of the clinical symptoms and the improvement appeared only when the drug doses went down. Although recurring knee symptoms with an increase in tacrolimus dose would be much stronger proof of this association, it is not ethically justifiable. Furthermore, she was treated with verapamil in addition to other drugs for con-
trolling hypertension. Verapamil might have played a part in a possible increased risk for this clinical complication, because it decreases tacrolimus clearance.5 However, there are reports that calcium channel block-

ers (albeit of the dihydropyridine type) can improve the bone pain syndrome.6 Although leg bone pain syndrome in kidney transplant patients who have received cyclosporin A is very rare, there are case reports described in the literature.7 To our knowledge, this is the first case of a renal transplant patient with pain in the lower limbs, related to tacrolimus treatment. Additional case reports are needed to support this association.

Spleen haemorrhagic infarction and hazards of anticoagulation in Wegener’s granulomatosis

In the largest cohort published to date, no spleen involvement is described in Wegener’s granulomatosis (WG).1 We report on two patients who required splenectomy for symptomatic spleen infarction in the course of WG.

CASE 1

A 42 year old man was admitted with an eight month history of arthritis and lower limb dysaesthesia. Examination showed an acutely ill patient with a 39°C fever, oral ulcers, haemorrhagic gingival hyperplasia, bilateral haemorrhagic nasal discharge with crusts, diffuse necrotic purpura, neuritis, and black discoloration of some fingers and toes. The spleen was not palpable. Silent anterior myo-
cardial infarction was diagnosed because of elevated MB-CK and ST-segment increase with loss of R waves in leads V1, V2, V3 on electrocardiogram.1 Anti-neutrophil cyto-
plasmic antibodies (c-ANCA) were disclosed in serum and necrotising vasculitis was shown on skin biopsy specimen.2 No antiphospholi-
bohydrate antibody or coagulant protein abnormality could be disclosed. Treatment con-
sisted of intravenous administration of prednisolone, cyclophosphamide, sodium heparinate, diltiazem, dinitrosofide and enalapril. His short-term course was uneventful. At day 14, the patient suddenly developed a severe haemorrhagic shock. Echotomography of the abdomen showed a splenic mass. At laparotomy, the spleen was almost disrupted by voluminous haematoma. Histological analysis of the spleen showed widespread necrotising vasculitis with haem-
orrhagic infarction. After five years of follow up, the patient is in complete remission with oral corticosteroid treatment.

CASE 2

A 23 year old young man was admitted in August 1996 because of repeated otitis media, sinusitis, epistaxis, headache, arthralgias with fever and weight loss. Despite a short course of oral corticosteroids and antibiotics, his general condition worsened. Antiproteinase 3 c-ANCA were disclosed in serum. Chest computed tomography showed pulmonary nodules. Intranasal endoscopic biopsies demonstrated necrotising vasculitis with epi-
theiloid and giant cells. Treatment included oral prednisone and intravenous cyclophos-
phamide pulses. After a few days, serum creatin-

ine concentrations abruptly increased to 198 μmol/l and urine analysis showed micro-
scopic haematuria and proteinuria. High dose methylprednisolone pulses were then given, intravenous cyclophosphamide was changed to 100 mg daily and diltiazem, the patient eventually achieved remission. In October 1996, abdomen computed tomograp-
hy showed an intrasplenic lesion that was consistent either with a splenic infarct or hae-
morragia (fig 1). The latter course was marked by a WG flare in January 1997, which was complicated with massive thrombosis of the left iliofemoral vein and the inferior vena cava. No thrombophilic disorder could be found. Intravenous heparin then oral anticoagulation with acenocoumarol were given. Because of persistent left hypocondriac tender-
ness, splenectomy was performed in Sep-
tember 1997. Histological examination showed spleen infarction with organised hem-
atomata and sequelae of vasculitis (fig 2).

COMMENT

Because they are vessels without collateral flow, occlusion of distal parenchymal splenic arteries leads invariably to infarction. Of note, two of the three patients described by Wegener in 1936 had spleen involvement.9 The frequency of spleen involvement ranges from 4–6%

References


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9 Kozin F, Ryan L, Carrera G, Soin J, Wortmann R. The reflex sympathetic dystrophy syndrome (RSDS): scintigraphic studies, further evidence for the therapeutic efficacy of systemic corti-


11 Redlich K, Pietschmann P, Sniele T, Peterlik M. Comparative study on the e

e-ect of calcium channel block-

ers (albeit of the dihydropyridine type) can
process. A severe splenic haemorrhage occurred in patient 1, which was clearly related to both necrotising vasculitis and hypocoeagulable state. Anticoagulation was indicated for an inaugural myocardial infarction in case 1 and deep venous thrombosis in case 2, in both cases during active WG flare. Splenectomy was required in both our cases. Our data suggest that antithrombotic treatment entails a specific risk of bleeding complications in patients with WG vasculitis. When anticoagulation is necessary in WG patients, computed tomography of the abdomen should be systematically performed and, if splenic infarction is disclosed, splenectomy should be considered.

Figure 2 Cut gross pathological section of spleen shows changes corresponding to those seen by computed tomography. Large firm yellow (white) area consistent with infarction was surrounded by dark peripheral zone of splenic parenchyma.
patients with DIL are usually older; the prevalence of men and women is similar, and the presenting symptoms are usually mild, with the patient usually complaining of malaise, fever and arthralgia, with or without arthritis, while skin, central nervous system or renal involvement is rare. Pleuropericardial disease is frequent and, as in classic SLE, anaemia and leucopenia may be present. Serum complement components are usually normal, ANAs are positive but anti-dsDNA and anti-Sm are negative, while anti-histones antibodies can be detected in most of patients. The pathogenic mechanisms proposed for DIL include: cross-reactivity between drug and the nucleic acid; hapten complex formation between drug and nucleic acid, or structural damage to the chromosomal DNA; action of a drug as an adjuvant or immunostimulant, which, in concert with appropriate immune response genes, triggers polyclonal B/T cell activation; and interference with the complement pathway. The incidence of side effects associated with amiodarone ranges from 40% to 93% and, in most of cases, these side effects are consequence of its potential to be directly toxic to several organ systems. However, there is also some evidence of immunologically mediated phenomena related to amiodarone. A positive skin and basophil degranulation test with amiodarone, secretion of leucocyte inhibitory factor, positive lymphoblastic transformation and circulation of a specific antibody of the IgG class have been described. Moreover, several studies suggest that various biological and immunological markers of “systemic” disease activity are present even in patients taking this drug. Circulating immune complexes, ANAs, and non-specific increase in ESR and white blood cell count, sometimes with eosinophilia, are common findings.

Low ANA titre is not uncommon in an elderly patient. However, spontaneous SLE in elderly people is not usual and DIL must always be considered in the differential diagnosis. This case, presenting with malaise, fever, arthralgia, circulating immune complexes, and autoantibodies strongly suggests an immunological underlying condition. Moreover, this patient meets four SLE criteria: malar rash, serositis, haematological and articular damage to the chromosomal DNA; and, in consequence, the immune response might progress despite discontinuation of the treatment.

Antinuclear antibodies in relapsing polychondritis

The prevalence of antinuclear antibodies (ANA) in relapsing polychondritis (RP) has been recently reported by Zeuner et al. The positivity in a literature review. The charts of 180 patients followed up in our institution fulfilling the criteria for RP proposed by Michet et al have been recently retrospectively reviewed, with special focus on dermatological manifestations and their relation with myelodysplasia. This aim led us to exclude 36 patients because the association of RP with potentially confounding diseases, such as systemic lupus erythematosus (SLE) present in nine, mixed connective tissue disease (MCTD) in five, rheumatoid arthritis in three, Takayasu arteritis in three, mesenteric panniculitis in three, spondylarthropathy in two, Crohn’s disease in two, psoriasis in two, or Lichten planus in two. Among the 144 patients remaining, 111 have been tested for ANA by using either HEP-2 cells or liver sections as substrate, or both. Most patients had repeated ANA determinations, including initial testing before onset of corticosteroid treatment, and the higher titres were considered for analysis. Figure 1 shows the histogram of ANA positivity according to ANA titre.

ANA were either absent or present in low titres in a majority of patients (73% and 18%, respectively). “Significant” titres—that is, titre > 1/100 ANA—were demonstrated in only 10 of 111 patients (9%). The pattern of fluorescence was as follows: homogeneous in five, speckled in two, speckled and nucleolar in one, perinuclear and nucleolar in one, and not provided in one. Among those 10 patients, five had clinical or ophthalmological features, or both, suggestive of an associated Sjögren’s syndrome including two who had antibodies to both SS-A and SS-B, and two others had a myelodysplastic syndrome.

None of these 10 patients had antibodies to ds-DNA. Using the same methods, ANA > 1/100 were found in 15 of the 36 patients initially excluded (42%). Among the nine with SLE, ANA > 1/100 and antibodies to ds-DNA (by Farr assay or Criehtiafluoride method) were found in eight patients. All five patients with MCTD had ANA > 1/1000 (in a speckled pattern in four), with positive antibodies to RNP and negative tests for ds-DNA. The two remaining patients with ANA > 1/1000 had either SS-A and SS-B, or Lichten planus associated with multinodular goitre; both of them also had features suggestive of sicca syndrome. Therefore, within these 36 patients, strong ANA positivity was more likely in patients with MCTD or Sjögren’s syndrome, or all three.

Beside the recent article by Zeuner et al, the prevalence of ANA has rarely been reported in RP. Michet et al have found positive ANA in four of their 18 patients tested (22%), and noted 3 of 23 (13%) ANA positivity in a literature review. Data regarding ANA were not provided in the large series of patients with RP followed up at the Mayo Clinic. The low prevalence of ANA observed in our cases with “pure” RP—that is, RP not associated with another connective tissue disorder except for a possible Sjögren’s syndrome—agrees with the negative results of tests for IgG antinuclear antibodies recently reported by our group in this condition.

We conclude that: (a) the prevalence of ANA observed in RP is low and, (b) as suggested by other authors, the finding of a significant titre of ANA in a patient with RP strongly suggests the presence of an associated disorder, such as SLE, MCTD, Sjögren’s syndrome or acquired myelodysplasia.

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There is no association between polyvalmy rheumatica and acute parvovirus B19 infection

Parvovirus B19 has been associated with a growing number of diseases. Besides the frequent manifestations such as erythema infectiosum, aplastic crisis in persons with underlying haemolytic anaemia, hydrops fetalis in pregnant women and acute or chronic arthritis a range of rather rare diseases have been described in recent reports. Among them are case reports on persistent parvovirus B19 infection in immune incompetent people, encephalitis, myocarditis, systemic lupus erythematosus (reviewed by Anderson and Youle) and rheumatoid arthritis. Furthermore, parvovirus B19 has been suspected to play a part in the aetiology of polyvalmy rheumatica (PMR). Because of the acute onset of PMR and its systemic symptoms an infection of the latter virus seems to be a relevant factor. Additionally, autoimmune processes have been demonstrated in both, PMR and parvovirus B19 infection. As the receptor for parvovirus B19, the F-blood group antigen (glycophorin), is also present on endothelial cells an interrelation between parvovirus B19 and giant cell arteritis or PMR may be possible.

Parvovirus B19 can only replicate in erythroid precursor cells in human bone marrow, but it is known that infection of cells non-permissive for viral replication leads to an excess production of the viral non-structural protein (NS1) without production of capsid protein VP1. As the NS1 protein is cytotoxic and able to induce apoptosis, it probably plays a part in the pathogenetic process of the parvovirus B19 induced tissue damage. This is confirmed by the fact that antibodies against NS1 of parvovirus B19 are preferentially produced during chronic or persistent parvovirus B19 infections, for example in parvovirus B19 associated chronic arthritis.

To test the hypothesis whether PMR is associated with acute parvovirus B19 infection, we tested the seroprevalence of IgG antibodies against the two structural proteins VP1 and VP2 and against the non-structural protein NS1 in 110 PMR patients (patients with giant cell arteritis excluded; mean (SEM) age 67.0 (0.8) years, range: 48–77) and, for comparison, in 135 healthy controls of different ages. At the time point of blood sampling (median disease duration at the time point of blood sampling was 0.6 years, range: 0–7.3, mean (SEM): 1.4 (0.2) years), 35 patients had no corticosteroids and 75 patients received on an average 15.2 (1.8) mg prednisolone/day. Furthermore, we investigated the association between age and symptoms or laboratory parameters and the presence of NS1 specific antibodies in healthy controls and patients with PMR. Non-parametric Kruskal-Wallis one way analysis was used to compare means of different subgroups. The significance level was p<0.05.

Subjects in the control group had various ages between 18 to 75 years. Overall seroprevalence of IgG against the capsid proteins VP1 and VP2 was 78% (fig 1). Overall IgG seroprevalence against VP1 and VP2 was 88% in patients with PMR (not significantly different versus the age matched control group). With respect to the NS1 IgG antibody, overall seroprevalence in the control group was 22% (fig 1) and in patients with PMR 20% (p=0.057 versus the age matched control group: 10%; fig 1).

Furthermore, we investigated the association between the presence of NS1 IgG antibodies and PMR related symptoms or laboratory parameters (patients with NS1 compared with patients without NS1 IgG antibodies were not different in age, sex, and medication). The symptoms were assessed using standard record forms from the medical history (at the time serum was collected). We asked the patients for muscular pain in the left/right shoulder, left/right upper arms, left/right neck, left/right gluteal muscle, and left/right thigh. If one muscle group was painful, the corresponding item was scored with one point (the sum of the item points was the overall muscle score). In PMR patients with NS1 IgG as compared with patients without NS1 IgG, arthralgia was more frequent (with versus without: 73% vs 40%, p=0.024). However, the overall muscle score was lower in NS1 positive than in NS1 negative patients (0.5 (0.2) SEM vs 1.6 (0.5) SEM score points; p=0.021). With respect to other PMR related symptoms, no significant differences were found. In patients with a positive NS1 IgG antibody, interleukin 6 (4.6 (0.9) SEM vs 11.3 (2.2) SEM; p=0.037) and soluble ELAM (48.2 (4.8) SEM vs 71.4 (5.2) SEM; p=0.024) were significantly lower as compared with patients without NS1 IgG. No significant differences were found with respect to erythrocyte sedimentation rate, C reactive protein, tumour necrosis factor, interleukin 2, and interleukin 10.

In view of these data, there was a positive association between NS1 and arthralgia. However, other symptoms and parameters of inflammation such as erythrocyte sedimentation rate were not associated with the presence of NS1 IgG. Furthermore, disease related immune mediators such as interleukin 6 or soluble ELAM were lower in patients with as compared with patients without NS1 IgG. As a positive NS1 IgG indicates an active infection, an acute parvovirus B19 infection does not seem to be a pathogenetic factor in our patients with PMR.

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References


Figure 1 Seroprevalence of IgG antibodies against VP1/VP2 (black bars) and NS1 (open bars) in control subjects and patients with polyvalmy rheumatica (PMR). The number of healthy controls was 22% and in patients with PMR 20% (p=0.057 versus the age matched control group: 10%; fig 1). With respect to other PMR related symptoms, no significant differences were found. In patients with a positive NS1 IgG antibody, interleukin 6 (4.6 (0.9) SEM vs 11.3 (2.2) SEM; p=0.037) and soluble ELAM (48.2 (4.8) SEM vs 71.4 (5.2) SEM; p=0.024) were significantly lower as compared with patients without NS1 IgG. No significant differences were found with respect to erythrocyte sedimentation rate, C reactive protein, tumour necrosis factor, interleukin 2, and interleukin 10.
Neutrophil chemotaxis in Behçet’s syndrome

It has been suggested that the marked cellular inflammatory response, which characterises Behçet’s syndrome (BS), may be attributable to increased neutrophil locomotion. However, others disagree. We have re-evaluated chemotaxis of polymorphonuclear leucocytes (PMNs) in BS among a greater number of patients in a controlled setting.

Fifty-four male BS patients, nine male patients with ankylosing spondylitis, eight with psoriasis and 37 male healthy controls were studied with 28 female patients with BS and 16 healthy female controls. Behçet patients with severe disease were those with active major vessel and/or eye involvement.

We measured chemotaxis with the “under the agarose method”. The measurements were masked with the assessors not knowing the diagnoses. An inverted microscope fitted with an ocular micrometre disc to measure the migration of neutrophils from middle wells to outer (chemotaxis) and inner wells (chemokinesis) was used. Zymosan activated sera (patients or controls) were used as a source of chemoattractants. Results were expressed as micrometre square (1 mm²=8 squares). Additionally the plates were evaluated macroscopically for observation of the migration between neutrophil wells. Tables 1 and 2 show the results. There were no significant differences between the chemotactic indices of the various groups of patients and controls studied or controlled by either sex. Maximal chemotaxis rates in the groups varied from 67% to 100%.

The Boyden millipore filter system has extensively been used for chemotaxis experiments. The agarose method is simple and cheap. This method can preferentially be used to differentiate chemokinesis from chemotactic migration.

There is marked heterogeneity in disease expression in men and women in BS and we recognised that some of the confusion in the literature about neutrophil activity might be related to this. Thus we analysed our data separately for either sex. Although there was a tendency for male patients with BS to have higher chemotactic indices this was not statistically significant (p=0.62). We did not study any diseased controls for female patients with BS.

Abdulla and Lehner observed decreased chemotaxis in BS. Fordham et al., on the other hand reported increased chemotaxis, but normal random migration. While Wilkinson, similar to our experience, observed normal chemotaxis in BS, more recently Carletto et al. reported augmented chemotaxis especially in the active phases of the disease. Finally, Ben Ezra et al., among a group of Behçet patients with uveitis could demonstrate increased chemotactic activity only among a few of these patients, compared with that observed among patients with other forms of uveitis. They concluded that increased chemotactic activity was not a regular feature of ocular BS (personal communication).

In vivo assays do not differentiate chemotaxis from chemokinesis. In the Carletto study clinically active Behçet patients demonstrated increased chemotaxis to sera by Senn’s modified in vivo assay. Others had found hyperchemotaxis to be a specific diagnostic feature again by using an in vivo assay. Although it is difficult to compare the results of in vitro and in vivo assays, we thought these reported increases might have resulted from increased chemokinesis. In our experiments we observed maximal chemotaxis (3 mm) frequently, however, we did not find any significant differences in chemotactic indices between diseased and healthy subjects.

An interesting aspect of our study was the migration between neutrophil wells that was observed in many of the Petri dishes. This was observed even though we had not used cellular materials as chemoattractants. Presumably the gravity of the cellular materials overcame the chemical gradient of zymosan activated sera in some Petri dishes. Because of the observed migration between neutrophil wells, we suggest that there should be only one “triple well rank” in a Petri dish. On the other hand our method of precinuation of the whole blood for 45 minutes at 37°C before harvesting the PMNs (intended for better viability) might have been responsible for this phenomenon by increasing the chemotactic activity in all groups studied. Further studies are needed to clarify these issues.

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**Table 1: Chemotactic indices in men**

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<th>Number</th>
<th>Mean (SD)</th>
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<td>54</td>
<td>6.4 (8.0)</td>
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<tr>
<td>Severe Behçet’s</td>
<td>9</td>
<td>11.3 (12.1)</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>9</td>
<td>5.9 (7.1)</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>8</td>
<td>10.8 (11.0)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>37</td>
<td>10.0 (10.3)</td>
</tr>
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*Kruskal-Wallis one way analysis of variance, correct for ties: $\chi^2(2) 1831$, DF=4, p>0.05.

**Table 2: Chemotactic indices in women**

<table>
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<th>Mean (SD)</th>
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<tr>
<td>Behçet’s</td>
<td>28</td>
<td>7.2 (8.2)</td>
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<tr>
<td>Healthy controls</td>
<td>16</td>
<td>10.1 (10.2)</td>
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Neuropsychiatric systemic lupus erythematosus

The considerable difficulties in making sense of the literature on patients with lupus involving the central nervous system are re-emphasised in the paper by Rood et al. The authors, who to be fair take a sensibly cautious approach to their results, nevertheless seek to persuade us that the IL10 locus is associated with neuropsychiatric lupus on the basis of a historical case note review of 42 lupus patients with neuropsychiatric disease, compared with 50 who lack such involvement.

Their conclusion needs to be treated with caution. Does it make sense to lump together 40 diverse patients and make the kind of claim they have made? The authors suggest that CNS lupus is attributable to either antiphospholipid antibody related thrombotic events, or “immune mediated” disease. This division is artificial. There is a considerable literature on CNS lupus that proposes that a wide variety of immunopathogenic mechanisms may be responsible in individual cases. These mechanisms include thrombotic effects, which may be linked to antiphospholipid antibodies, a true vasculitis, a cross reaction between antibodies that recognise the lymphocyte surface targets and neurochemical antigens, and antibodies to a wide variety of neurological targets. A considerably larger number of patients will have to be studied before any claims of links to an IL10 promoter haplotype can be truly convincing.

We agree with the authors that patients with SLE have a higher innate production of IL10 than controls. However, as there is no significant difference in the frequency of the IL10 promoter single nucleotide polymorphism (SNP) in SLE patients when T, Barnes CG, eds. Recent advances in Behçet’s disease. London: Royal Society of Medicine Services, International Congress and Symposium Series, no 103, 1996:97-8.


compared with controls in their study, we suspect that the difference in IL10 production in patients with SLE and controls in terms of the IL10 SNP alleles frequencies is not attributable to functional differences between patients with SLE and controls. Differences have been described with respect to microsatellites, and one awaits confirmation from other populations or family studies. To our knowledge, a difference in IL10 production between patients with neuropsychiatric disease SLE and non-neuropsychiatric disease SLE has not been described. The described associations would be biologically meaningless if IL10 production is similar between these two groups.

The authors suggest that the -1082A allele is associated with a higher innate IL10 production, however, they appear to ignore the only published study to date that showed that the A allele was associated with lower IL10 production. In addition we have confirmed that the A allele is associated with lower IL10 production in transient transfection studies and the ATA/ATA genotype is associated with lower IL10 production in whole blood culture. The increase in the A allele is mainly accounted for by an increase in the ATA haplotype in their neuropsychiatric disease patients and therefore they are described with a lower IL10 producing haplotype, not a high IL10 producing haplotype. One interpretation of this would be that patients with neuropsychiatric disease symptoms are unable to adequately control inflammation from a variety of different pathological mechanisms because of low IL10 production.

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Authors’ reply

We thank Drs Isenberg, Cawley and Woo for their interest in our paper. They argued that the dichotomy of the pathogenesis of CNS lupus in “immune mediated” and thromboembolic disease is too rigid, as both pathogenic mechanisms can be deemed responsible for CNS lupus. As the hallmark of SLE is the production of autoantibodies, it seems to be justified to assume that the pathogenicity of CNS lupus is B cell mediated. Based upon this assumption we clustered the individual neuropsychiatric disease SLE patients and tested the hypothesis that a genetic marker in the promoter of the IL10 gene is associated with the phenotype of CNS-SLE.

In general, a positive result in a genetic association study is only possible after a correct definition of the phenotype. After all, if the phenotype is not clearly defined, the magnitude and statistical significance of the association will be less or lost because of the random distribution of the genetic marker in the misclassified patients. If misclassification occurred in the sense that CNS lupus patients were attributed to the non-neuropsychiatric disease SLE population, the fact that we still found a positive result strengthens our conclusions instead of weakens it.

It might be argued that thromboembolic events do not fit in the pathogenic model of B cell mediated CNS lupus. But, as stated clearly in the article, even after exclusion of these ambiguous patients, the distribution of the frequencies in the neuropsychiatric disease SLE and non-neuropsychiatric disease SLE patients remains the same.

Of course we agree with the notion that our findings must be repeated in another group of patients. Interestingly, the increased prevalence of ATA in neuropsychiatric disease SLE patients has already been reported by Mok in a group of Chinese SLE patients. Currently we are investigating the distribution of the IL10 promoter haplotypes of neuropsychiatric disease SLE patients in an ethnically different population.

In our article we have elaborated on two possible explanations of our findings. Firstly, the increased frequency of the ATA haplotype might be associated with an increased production of IL10. We made this assumption in the light of previous studies stating that SLE as a whole is characterized by an increased innate IL10 production.7 It is wrong to extrapolate these conclusions to our population. Because of the retrospective character of our study, we were not able to measure IL10 production in our populations and therefore cannot say whether or not IL10 production in our SLE patients as a whole was similar to or different from the control population. It might well be that differences in IL10 production would only emerge after stratifying into neuropsychiatric disease SLE and non-neuropsychiatric disease SLE patients. Furthermore, it might be that in the populations mentioned before, there was an excess of patients with neuropsychiatric disease SLE.

The second explanation for the skewing found in IL10 promoter polymorphisms might be the indirect susceptibility to neuropsychiatric disease SLE in the ATA patients is not conferred via an increased IL10 production at all, but that it is merely a marker for the real neuropsychiatric disease SLE susceptibility allele. It is not clear whether or not IL10 promoter SNPs are associated with low or high IL10 production, because of the ambiguous reports in the literature. In our laboratory, the -1082 A allele has been found to be associated with high IL10 production.9 In this light we have speculated that the possible link of high IL10 production and the pathogenesis of neuropsychiatric disease SLE. Iserenberg et al have referred to another group stating that -1082 A is associated with a lower IL10 production and they interpret our results with this finding in mind.9 In conclusion, we do not know the relevance of the IL10 promoter in the in vivo regulation of IL10 production and therefore both explanations are equally speculative.

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References


A man with intermittent fever and arthralgia

Knight and Symmons report a very interesting case of a man with Whipple’s disease and provide us with a timely update on this rare condition. They describe how six months after initial presentation the diagnosis of the adult-onset Still’s disease (AOSD) was made and despite regular review at several centres, this diagnosis was upheld for a further five years. Multiple investigations were performed adding little to the original diagnosis. It is not mentioned whether a serum ferritin was taken. This may have been useful given the initial diagnosis of AOSD as it might have resulted in questioning this diagnosis, permitting an earlier diagnosis of Whipple’s disease. It is widely reported in the literature, as far back as 1975, that increased serum ferritin may be of use in both diagnosis and monitoring of AOSD.4,5 Hyperferritinaemia is not however exclusive to AOSD, as various malignancies, hepatic necrosis and haemachromatosis can all cause it. However, values up to 25 000 µg/l have been recorded in AOSD and reportedly, values rarely exceed 3–5000 µg/l in the above conditions. The pathogenesis is not clearly understood, but it has been hypothesised that in AOSD, cytokine upregulation of ferritin mRNA...
translational control of ferritin synthesis in haemachromatosis and iron overload syndromes. A comparative study of diagnostic criteria in AOSD by Mason et al suggest the Yamaguchi criteria are superior to the others tested, including Cush et al quoted by Knight and Symmons. However, none of the criteria to aid diagnosis make use of serum ferritin measurement despite the claims for its use in the literature and acceptance in clinical practice. Although undoubtedly useful if very high, it is not clear what the relevance of a normal value in AOSD is, in a case satisfying clinical diagnostic criteria (although we have never seen such a case). In rare diseases such as AOSD, it is difficult to assess and evaluate diagnostic criteria and calculate sensitivity and specificity of possible disease markers. If serum were stored on this patient it would be interesting to know the serum ferritin measurement and how, if at all, it would have affected this patient’s management.

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Authors’ reply

We thank Drs Quinn and Gough for their interest in our paper. Our patient did have his serum ferritin measured in 1992. It was 197 μg/L (normal range 15–200). This was therefore a situation in which the patient satisfied the clinical diagnostic criteria for adult onset Still's disease (AOSD) but had a normal ferritin concentration. As the authors point out, had the ferritin concentration been high, this could not actually be used to refute the diagnosis. It was always felt that this patient’s disease was not typical of AOSD and the various physicians who looked after the patient were always willing to consider alternatives. However, it is difficult, even with the benefit of hindsight, to conclude that Whipple's disease could have been diagnosed earlier. Although the normal serum ferritin was not in keeping with the diagnosis of AOSD it did not point towards any other diagnosis in particular.

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Ear, ear, what’s going on in Norfolk?

Having recently started work in the rheumatology department of the Norfolk and Norwich Hospital I read with great interest the article on Hugh(h)e(s)’ ears: an unusual presentation.1 Amazingly we have recently seen an almost identical, but less catastrophic case.

A 27 year old white man presented with a 24 hour history of ears so swollen and painful, that he could not lie in bed with them touching the pillow. One year previously he was diagnosed with primary antiphospholipid syndrome (APLS) after recurrent deep vein thromboses and a raised IgG antiphospholipid antibody at 92 iu/ml. He was subsequently given warfarin.

On admission this time, his INR, while taking warfarin 7 mg per day, was subtherapeutic at 1.6. Biopsy from his left ear lobe showed numerous fibrin thrombi with no associated vasculitis, consistent with thrombosis secondary to APLS.

His warfarin dose was increased to obtain an INR between 3 and 4. Within a few days he had recovered and was discharged home well.

Perhaps Hughes’ ears should be renamed Norfolk ears?

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Authors’ reply

We note with interest the report from Dr Gaffney. While the coincidence is indeed curious, these cases do suggest possible mechanisms for activation of thrombosis.

The external ear is characterised particularly by a lower average temperature than core body temperature, and by its susceptibility to trauma and pressure effects. In our case, cryoglobulins were not identified, and no comment in this regard is made by the authors. There is no specific reference to any aural trauma, though presumably, as in our case, it is difficult to assess what pressure was exerted on the external ear during sleep. It is plausible that such pressure causes a degree of blood stasis, which together with inadequate anticoagulation, resulted in thrombosis. Such speculation may be interesting, but it is this latter point that deserves emphasis—patients with antiphospholipid syndrome who have had thrombus will do so again, potentially with serious consequences, if the INR is not scrupulously maintained above 3.0, a message that must be spread widely: “Friends, Norfolk countrymen, lend me your ears!”

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