Protein kinase signals activate interleukin 16 encoding transcripts in rheumatoid arthritis versus osteoarthritis synovial fibroblasts

M K Schuler, S Sell, W K Aicher


Interleukin 16 (IL16) is a proinflammatory cytokine and a chemoattractant factor for CD4+ T cells. IL16 has been detected at higher concentrations in rheumatoid arthritis (RA) synovial fluid than in osteoarthritis (OA) specimens. IL16 is expressed in inflammatory infiltrates and in CD68+ synovial lining cells of patients with RA as detected by in situ hybridisation.

In this study we compared the modulation of IL16 steady state mRNA in synovial fibroblasts (SF) from six patients with RA and from three patients with OA. SF were prepared, expanded, and characterised as described previously. To examine the IL16 encoding transcript amounts, SF were incubated in complete medium for 24 or 48 hours in the presence of one of the following chemicals: 1 ng/ml phorbol-12-myristate-13-acetate (PMA), an activator of protein kinase C (PKC); 200 ng/ml ionomycin (Iono), a calcium ionophor; 10 µM of adenosine-3',5'-cyclic monophosphate (cAMP), which stimulates protein kinase A (PKA); 10 nM okadaic acid (Oka), a phosphatase inhibitor; 10 µM MAS-7, which activates G-proteins; 100 µM H-7 dihydrochloride (H-7), an inhibitor of protein kinases; and 10 nM staurosporine (Stauro), a protein kinase inhibitor (all from Calbiochem or Biomol). Differences of IL16 encoding steady state mRNA amounts in activated cells compared with controls were detected after 33 cycles of reverse transcriptase-polymerase chain reaction (RT-PCR) amplification (Taq DNA polymerase, Roche Biochemicals). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RT-PCR served as a control for RNA content. The PCR amplification plateau was reached after 35 cycles. This suggested that the IL16-specific RT-PCR was suitable for detecting different levels of IL16 encoding transcripts as the PCR was stopped before reaching the amplification plateau. Still, the limitations of this method are evident and we therefore consider our data as a semiquantitative enumeration of transcripts encoding IL16.

Both, early passage RA SF and OA SF spontaneously transcribed IL16 encoding mRNA. Addition of protein kinase inhibitor staurosporine enhanced the IL16 RT-PCR signals in all samples of OA SF, whereas specific protein kinase C activator PMA reduced the IL16 encoding RT-PCR signals in OA SF (fig 1). Ionomycin, cAMP, and MAS-7 had minor and variable effects in OA SF (fig 1). Addition of protein kinase inhibitor staurosporine also enhanced the IL16 encoding signal in RA SF (fig 2). Incubation of the cells with PMA and ionomycin reduced the IL16 encoding RT-PCR signal intensity in these cells (fig 2). Again, cAMP and MAS-7 produced minor and variable effects in the different samples analysed (fig 2). Application of okadaic...
Obstructive sleep apnoea as a cause of fatigue in ankylosing spondylitis

N Erb, D Karokis, J P Delamere, M J Cushley, G D Kitas

Fatigue is a common symptom in ankylosing spondylitis (AS) occurring in 65% of patients and forms part of the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). Fatigue has been attributed to sleep disturbance from back pain and stiffness and usually increases with increased disease activity, but can occur independently of AS activity, suggesting the possibility of other causes. One such cause in the middle aged population is sleep apnoea syndrome (SAS). SAS is defined as 10 or more episodes an hour of airflow interruption for ≥10 seconds during sleep. It occurs in up to 4% of middle aged people and is associated with increased morbidity and mortality due to higher rates of cardiovascular disease and increased accidents.

We suggest that AS predispose subjects to SAS through several mechanisms, including: restriction of the oropharyngeal airway from temporomandibular joint involvement or cervical spine disease causing pharyngeal and tracheal compression (as has been described in rheumatoid arthritis); cervical spine disease causing compression of the respiratory centres in the medulla resulting in central depression of respiration; or restrictive pulmonary disease. We carried out an observational study to assess the prevalence of SAS, and to investigate whether it contributes to fatigue in AS.

PATIENTS AND METHODS

Consenting volunteers with classical AS (modified New York Criteria 1984) were recruited prospectively from a hospital rheumatology clinic and assessed using: (a) the BASDAI; (b) the Eworth Sleepiness Scale (ESS), a validated self administered eight item questionnaire that assesses daytime sleepiness in adults (a score of ≤10 is normal); (c) the Hospital Anxiety and Depression Scale (HAD) (a score of ≤7 indicates normal mood); (d) height, weight, neck circumference; (e) spinal mobility by occiput-wall distance, chest expansion, and Schöber’s test; (f) respiratory measurements consisting of full spirometry and carbon monoxide diffusion studies, arterial blood gases, and night oximetry on two consecutive nights (using a five channel EdenTec Recorder and EdenTrace Software Version 1.3, Nellcor, Puritan and Bennett, Ltd) to assess heart rate, chest impedance, nasal airflow, oxygen saturation, and snoring level.

RESULTS

Of 22 recruited patients, 17 (77%) completed the assessments, 14 male and three female. Pulmonary function testing was normal in nine (53%) patients, classically restrictive in six (35%), borderline restrictive in two (12%), and obstructive in none. Two (12%) patients fulfilled criteria for SAS when
assessed by night oximetry. Both these patients had an obstructive type of SAS (table 1).

Compared with those without SAS, the two patients with SAS had significantly higher mean ESS scores (SAS 16.5 (0.7) vs no SAS 8.6 (5.1), p<0.01), fatigue component of the BASDAI (SAS 8.0 (0.7) vs no SAS 5.8 (2.5), p=0.04), and neck circumference (SAS 41.0 (4.2) vs no SAS 39.2 (9.2), p=0.02). The overall BASDAI scores (SAS 5.5 (1.3) vs no SAS 4.83 (1.9), p=0.59) and body mass index (SAS 32.7 (3.1) vs no SAS 25.6 (4.6), p=0.13) were not significantly different between the two groups. Neither of the two patients with SAS drank alcohol, but no other significant differences were found between the two groups (table 1).

DISCUSSION
SAS and AS can coexist. We found a higher prevalence of SAS in patients with AS (12%) than has been reported in the general population (1–4%). However the sample size was small and a larger study would be required to determine the true prevalence. As might be expected, the patients with SAS had high subjective scores of daytime sleepiness, which was mirrored by the high scores on the fatigue component of the BASDAI. The overall BASDAI scores of the patients with SAS were not significantly different from the remainder of the cohort, suggesting that disease activity in these two patients did not differ from that of the cohort, and the high fatigue component scores were rogue results reflecting the underlying SAS and not AS activity. None of the specific measurements of spinal involvement in the affected patients were significantly different from those of the cohort, suggesting that the degree of spinal involvement in AS was not a contributing factor in the development of SAS in these two subjects. The two affected patients were both obese middle aged men and had a classical restrictive pattern on pulmonary function testing, all of which are known to be risk factors for the development of SAS. Both patients were treated with continuous positive airway pressure ventilation at night, and their levels of fatigue improved subjectively, which was reflected in a fall of their ESS scores (patient 1: 17 to 9, patient 2: 22 to 12).

SAS can be a contributing factor to fatigue in AS. Patients with excessive fatigue or scoring high on the fatigue component of the BASDAI without other evidence for continuing disease activity should be assessed for other causes of fatigue. Detection and treatment of SAS can lead to improvement in fatigue symptoms in these patients and reduce the associated morbidity and mortality of SAS.

ACKNOWLEDGEMENTS
Many thanks to the staff of the Pulmonary Function Laboratories in the Dudley Group of Hospitals NHS Trust for carrying out the respiratory tests and sleep studies.

REFERENCES

Table 1 Results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total group n=20 (SD)</th>
<th>No SAS n=15 (SD)</th>
<th>t Test (p)</th>
<th>SAS n=2 (SD) Patient 1 Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>16.1 (12.8)</td>
<td>16.9 (13.5)</td>
<td>0.76</td>
<td>49.0 (7.1) 54</td>
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<tr>
<td>Disease duration (years)</td>
<td>26.2 (13.3)</td>
<td>26.1 (14.2)</td>
<td>0.86</td>
<td>27.0 (4.2) 30 24</td>
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<td>BASDAI</td>
<td>4.9 (1.8)</td>
<td>4.8 (1.9)</td>
<td>0.59</td>
<td>5.5 (1.3) 6 6</td>
</tr>
<tr>
<td>Fatigue component of BASDAI</td>
<td>6.0 (2.5)</td>
<td>5.8 (2.5)</td>
<td>0.04</td>
<td>8.0 (0.7) 8 5</td>
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<tr>
<td>HAD</td>
<td>5.3 (3.0)</td>
<td>4.9 (2.9)</td>
<td>0.35</td>
<td>8.0 (2.8) 6 10</td>
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<tr>
<td>ESS</td>
<td>9.7 (5.5)</td>
<td>8.6 (5.1)</td>
<td>&lt;0.01</td>
<td>16.5 (0.7) 17 22</td>
</tr>
<tr>
<td>Alcohol (units/week)</td>
<td>6.7 (8.9)</td>
<td>7.5 (9.1)</td>
<td>0.01</td>
<td>0.0 (0.0) 0 0</td>
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<tr>
<td>Smoking (cigarettes/day)</td>
<td>6.7 (8.7)</td>
<td>6.2 (8.4)</td>
<td>0.77</td>
<td>10.0 (14.1) 0 20</td>
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<tr>
<td>Body mass index</td>
<td>26.4 (5.0)</td>
<td>25.6 (4.6)</td>
<td>0.13</td>
<td>32.7 (3.1) 30 34.9</td>
</tr>
<tr>
<td>Neck circumference (cm)</td>
<td>39.4 (8.7)</td>
<td>39.2 (9.2)</td>
<td>0.02</td>
<td>41.0 (4.2) 38 44</td>
</tr>
<tr>
<td>Occiput wall (cm)</td>
<td>5.9 (5.0)</td>
<td>5.5 (4.4)</td>
<td>0.71</td>
<td>9.0 (9.9) 2 16</td>
</tr>
<tr>
<td>Schobers (cm)</td>
<td>4.8 (4.0)</td>
<td>5.1 (4.2)</td>
<td>0.23</td>
<td>3.0 (1.4) 4 4</td>
</tr>
<tr>
<td>Chest circumference (cm)</td>
<td>2.9 (1.8)</td>
<td>3.1 (1.8)</td>
<td>0.09</td>
<td>1.5 (0.7) 2 1</td>
</tr>
</tbody>
</table>

SAS, sleep apnoea syndrome; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; HAD, Hospital Anxiety and Depression Scale; ESS, Epworth Sleepiness Scale.
Thoracic high resolution computed tomography in patients with ankylosing spondylitis and without respiratory symptoms

A El Maghraoui, S Chaouir, A Bezza, F Tabache, A Abouzahir, D Ghafir, V Ohayon, M I Archane

The incidence of pleuropulmonary disease in ankylosing spondylitis (AS) varies from 0 to 30% in the medical literature. The most frequently recognised manifestations are upper lobe fibrosis, mycetoma formation, and pleural thickening. The advent of high resolution computed tomography (HRCT) made it possible to examine the entire lung parenchyma and pleura in many conditions with diffuse lung disease by a non-invasive method.

Consecutive patients with a diagnosis of AS according to the modified New York criteria who attend our department during one year were included in the study. All patients had a prospective rheumatological assessment conducted by two rheumatologists (AEM and AB) using a structured questionnaire, a pulmonary function testing measurement, posteroanterior chest radiography; on the same day an HRCT of the thorax was performed using a Siemens Somatom S CT scanner with images windowed to highlight both lung and mediastinal structures. Nine HRCT slices were obtained on suspended respiration at 2 cm intervals from the lung apices to bases. The results of the chest radiographs and HRCT were assessed by a radiologist (SC) who was unaware of the clinical data of the patient. The CT scans were evaluated for the presence, distribution, and extent of airway and parenchymal abnormalities. Standard CT criteria were used to establish a diagnosis of interstitial lung disease (ILD), bronchiectasis, and emphysema.

Plain radiography was abnormal in only two patients. Twenty four patients (55%) showed abnormalities on HRCT. Table 1 lists the abnormalities detected on HRCT. Twenty (45%) patients had mild non-specific interstitial abnormalities of insufficient severity or extent to be labelled as ILD. Pulmonary function tests showed a restrictive process in eight patients, in whom three had normal chest HRCT and three had ILD. The two remaining patients had non-specific interstitial abnormalities (blebs, pleural thickening, parenchymal bands). Two patients had an obstructive process: one had normal chest HRCT and the other emphysema and apical fibrosis.

Our study disclosed a great percentage of defined as well as mild and non-specific interstitial abnormalities on HRCT undetectable on plain radiography in a series of patients with AS and without history of respiratory symptoms. Only one patient had evidence of ground glass shadowing, which is associated with active alveolitis (fig 1). This is usually considered a feature of early and potentially reversible disease. As previously described, the overall correlation of pulmonary function with radiographic appearance was poor. Casserly and Fenlon studied 26 patients with AS using HRCT and noted pulmonary abnormalities in 19 patients (73%). Findings consisted of interstitial lung disease (four patients), bronchiectasis (six patients), emphysema (four patients), apical fibrosis (two patients), mycetoma (one patient), and non-specific interstitial lung disease (12 patients). In that study plain radiographs revealed abnormalities in four patients. In contrast with our study, all patients with ILD had respiratory symptoms.

Another study conducted by Turetschek et al showed that 15/21 (71%) patients had abnormalities on thin section CT. The most common abnormalities were thickening of the interlobular septa (7/21 patients), mild bronchial wall thickening (6/21), pleural thickening and pleuropulmonary irregularities (6/21), and linear septal thickening (6/21). The HRCT findings in our study, as was the case in the study of Casserly et al, suggest an inflammatory process rather than a mechanical cause for the interstitial disease found in patients with AS. Twenty patients (45%) in our study had non-specific interstitial abnormalities as had 11 (42%) patients in the study of Casserly et al which implied HRCT evidence of interstitial change that was of insufficient severity or extent to be labelled as ILD. The significance of such changes is unknown and must await a prospective longitudinal study to determine their natural history.

Table 1 Results of chest HRCT in 44 patients with anklosing spondylitis

<table>
<thead>
<tr>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>Emphysema</td>
</tr>
<tr>
<td>Interstitial lung disease</td>
</tr>
<tr>
<td>Upper lobe fibrosis</td>
</tr>
<tr>
<td>Bronchiectasis</td>
</tr>
<tr>
<td>Ground glass attenuation</td>
</tr>
<tr>
<td>Non-specific interstitial change</td>
</tr>
<tr>
<td>Pleural thickening</td>
</tr>
<tr>
<td>Parenchymal bands</td>
</tr>
<tr>
<td>Subpleural bands</td>
</tr>
<tr>
<td>Blebs</td>
</tr>
<tr>
<td>Parenchymal micronodules</td>
</tr>
<tr>
<td>Irregular interfaces</td>
</tr>
</tbody>
</table>

Chest HRCT of a 37 year old patient showing ground glass aspect (black arrow) with parenchymal band (white arrow).
Effect of low dose weekly methotrexate on bone mineral density and bone turnover

S Patel, G Patel, D Johnson, L Ogunremi, J Barron


W

ijands and Burgers recently reported on a patient with psoriasis who developed stress fracture of the leg thought to be related to methotrexate treatment.1 The role of methotrexate in the aetiology of fractures remains uncertain, because nearly all subjects reported had other risk factors for fracture, such as the underlying disease being treated. We recently examined a group of patients with psoriasis (without features of arthritis, to avoid the bias which may occur due to the arthritis and compensatory action of methotrexate on inflammatory disease activity) to assess the effect of methotrexate on bone density and turnover.

After approval from the local ethics committee, patients with psoriasis, but without psoriatic arthritis or diseases or drug treatment known to adversely affect the skeleton, were recruited. We obtained information by interview and measured bone mineral density (BMD) by dual x-ray absorptiometry (DXA) using a Lunar DPX device (Lunar Corp, Madison, WI). Daily calibration measurements using an external phantom were performed and monitored for machine drift. No significant drift was noted during the study period. Precision was calculated by the method of Gluer et al.;2 and at our centre is 1.3% for the lumbar spine and 1.8% for the femoral neck. Morning samples of blood and second void urine were taken for biochemical analysis. Data are presented as mean (SD) unless stated. The significance of differences between groups was tested using paired and unpaired Student’s t tests where appropriate. One sample t test was used to determine if age adjusted BMD (Z scores) were significantly different from the densitometer control database. Correlations were examined using linear regression. A value of p<0.05 was considered significant.

Baseline assessments were performed on 30 patients, and 20 subsequently agreed to have repeat bone densitometry a mean of 21 months (range 17–24) later. The patients comprised 12 men and 18 women with a mean age of 56 years (range 32–85). Of the 18 women, 10 were postmenopausal (mean duration 21 years, range 3–39). All patients had been treated with methotrexate for a median duration of 2.0 years (interquartile range (IQR) 1.4–5.6). The cumulative median dose was 1387 mg (IQR 654–2250) and the weekly median dose was 9.8 mg (IQR 6.8–15.1).

Bone density was normal at the lumbar spine and femoral neck at baseline. Lumbar spine BMD was 1.205 (0.215) g/cm², the T score was −0.09 (1.98), and the Z score 0.833 (1.703). Respective values for the femoral neck were 0.938 (0.174) g/cm², −0.654 (1.463), and 0.224 (1.109). BMD did not change significantly from baseline in the 20 patients who participated in the longitudinal phase of this study. There was no relationship between weekly or cumulative methotrexate dose and change in BMD over this period of time. Baseline biochemistry of the patients was normal including parathyroid hormone and markers of bone turnover. There was no significant correlation between the duration of methotrexate use or dose (weekly and cumulative), BMD or markers of bone turnover. There were no differences in BMD Z scores for either skeletal sites or bone markers when women were classified according to menopausal status. Similarly the sex of the subject did not affect BMD Z scores or bone markers.

We report the effects of methotrexate on BMD and bone turnover at baseline and over two years in patients treated with methotrexate for psoriasis. We found that the prevalence of osteoporosis was no greater than would be expected for the age of the patient (Z scores were normal) and that for most patients, markers of bone turnover at baseline were within the normal range. Also no change in BMD was found when a subgroup of 20 patients were followed up prospectively. Bone turnover was normal and there was no change in BMD with chronic treatment. The rationale for choosing the patients studied was to avoid any confounding effects of underlying disease such as rheumatoid arthritis, which can itself cause local and systemic osteoporosis and abnormal bone turnover.3 None of our patients had systemic inflammatory disease and a recent study confirms that chronic psoriasis is not associated with osteoporosis.4 In our study BMD was measured at the standard skeletal sites for the diagnosis of osteoporosis. We did not measure BMD at sites of stress fracture reported with methotrexate,
Evaluation of a screening tool for inflammatory joint disease

J A Barbour, J Binding, M Bridges, C Kelly

The benefit of early treatment of inflammatory joint disease (IJD) with disease modifying drugs (DMARDs) to avoid progressive irreversible joint damage is well established. The time delay from onset of symptoms to starting a DMARD is determined by a number of factors, and early synovitis clinics have been developed to facilitate speedy referral and initiation of DMARD treatment. The efficiency of these clinics is dependent on appropriate referral. Diagnosing early IJD is not easy; even specialists have been shown to disagree when asked to diagnose the same patient. Therefore an effective screening tool could be used to maximise identification of patients likely to have IJD and minimise unnecessary use of urgent appointments.

A simple eight point questionnaire (box 1) was devised to separate patients with and without IJD. It uses well recognised diagnostic criteria for rheumatoid arthritis (morning stiffness, rheumatoid factor, and erosions) but also includes more general markers of inflammation (erythrocyte sedimentation rate (ESR)), benefit from non-steroidal anti-inflammatory drugs (NSAIDs)/steroids, synovitis, and family history.

We prospectively studied 100 consecutive patients whom their general practitioner (GP) suspected might have IJD and had referred to one consultant for early assessment over a 10 month period. GP letters were initially screened by the consultant and then passed to the nurse practitioner who applied the questionnaire (box 1) to all patients before the consultant's assessment.

Characteristic distribution for IJD was positive if more than one joint was affected by pain or stiffness, but negative if the pattern affected predominately the distal interphalangeal joints of the hands or the base of the thumbs. Synovitis was defined as the affected joint being tender and swollen. The most recent ESR was used, radiographs of hands and feet were used for assessment of erosions, and benefit from NSAID/steroids was taken as a reported patient global assessment. The diagnosis was taken as that made by the consultant at the first assessment. In cases where there was some doubt, it was taken as the most likely diagnosis at the subsequent review appointment. Seventy six women and 24 men not known to have IJD were included with mean ages of 55 years (women) and 50 years (men). The consultant diagnosed 31 as having IJD, of whom 30 scored 3 or more on the questionnaire (27 rheumatoid arthritis, 2 psoriatic arthritis, 1 palindromic

**REFERENCES**

Anti-annexin V antibodies in patients with cerebrovascular disease

N Gašperšič, U Rot, S Čučnik, T Kveder, B Božič, B Rozman


Annexin V (ANXV) is a protein with a high affinity for negatively charged phospholipids and shows in vitro a potent anticoagulant activity. It has been suggested that it has a significant role in the prevention of arteriovenous thromboses or fetal loss, or both.¹ Increased levels of antibodies against ANXV (aANXV) have been reported in patients with different systemic autoimmune disorders²⁻⁴ as well as in women with recurrent fetal loss and pre-eclampsia.³ The presence of aANXV in patients with thromboembolic cerebrovascular disease (CVD), however, has not yet been described. We report on two patients with CVD who had evidently raised levels of IgG aANXV, whereas all the other tested antiphospholipid antibodies (aPL) were negative.

We examined 37 young patients with no evident systemic autoimmune disease (23 women, 14 men; mean age at CVD 32 years (range 18–40) 11 months to six years after CVD: seven with transient ischaemic attack (TIA), 25 with ischaemic cerebrovascular insult, and five with venous sinus thrombosis. Diagnoses based on the history and clinical manifestations were objectively verified by computed tomography (CT), magnetic resonance imaging (MRI), and/or angiography at the time of the onset of symptoms. After prospective clinical re-examination, two blood samples were obtained from each patient eight weeks apart.

Serum samples were analysed by enzyme linked immunoabsorbent assay (ELISA) for the presence of aANXV, antiphosphodiesterase, anti-β₂-glycoprotein, and anti-prothrombin antibodies.⁵ Antinuclear antibodies (ANA) were determined by indirect immunofluorescence.

**CASE REPORTS**

**Patient 1**

A 36 year old woman with a history of fetal loss in 1982 became pregnant for the second time in 1998. At the 36th gestation week a caesarean section was performed owing to placental abruption. A few days after the delivery, she became somnolent with mild right sided hemiparesis. CT and an MRI scan confirmed superior sagittal sinus thrombosis and therefore treatment with warfarin was started. Three years later, her condition was stable with mild occasional headaches and mild right sided pyramidal symptomatology. Laboratory examinations showed positive ANA (up to 1/320) and persistently raised levels of IgG aANXV, while all the other tested aPL were negative. No clinical manifestations of a systemic autoimmune disease could be found. Except for a short period of smoking, no other thrombotic risk factors were identified.

**Patient 2**

A 24 year old woman had a TIA in 1996, two months after starting hormonal contraceptives. She experienced paraesthesia over both arms and legs and gait ataxia was found. MRI, echocardiography, and sonography of the neck vessels were normal, suggesting TIA in the vertebrobasilar region. In 2000 she became pregnant for the first time. Generalised oedema and hypertension appeared in the fifth and eighth month, respectively. A healthy child was born one month pre-term. In 2001 she was in good health except for rather frequent headaches. Clinical and special neurological examinations were completely normal. Among the tested aPL only IgG aANXV were found to be positive. Contraceptives were the only risk factor for CVD.

**DISCUSSION**

ANXV is one of the possible cofactors for aPL. Rand et al. reported that aPL can disrupt the protective shield of ANXV on procoagulant surfaces,⁶ leaving sufficient space for the formation of coagulation complexes. aANXV were shown to induce the apoptosis of endothelial cells, creating a procoagulant environment⁷ with increased risk for thrombosis.

Two of 37 young patients after CVD had significantly raised IgG aANXV only. Besides some CVD risk factors (smoking, delivery and bleeding, oral contraceptives) both patients had pregnancy complications, which might be associated with aANXV.⁸ Our results did not show a statistically significant association between aANXV and CVD. Nevertheless it is possible that aANXV represented an additional risk factor, and together with other factors might have led to thrombosis. A study of larger groups of patients will enable firm conclusions to be drawn about the clinical significance of aANXV in CVD.
Temporal arteritis (TA) is the most common systemic vasculitis. We report herein a case of TA complicated with intra-alveolar haemorrhage. To our knowledge, this manifestation has not previously been reported.

CASE REPORT

A woman born in 1926 presented in 1999 with persistent dry cough. Her medical history also included diabetes mellitus type II, hypertension, chronic lung disease, and arthritis. Physical examination was normal.

Physical examination was normal. Arterial pressure was 140/70 mm Hg. Leucocyte count was 6.8 x 10^9/l comprising macrophages 46%, neutrophils 31%, lymphocytes 10%, and platelets 30 x 10^9/l. A dipstick urinary test showed no proteinuria and no haematuria. Chest radiography disclosed calcified nodular opacities. She denied any visual or musculoskeletal manifestations. She had no haematuria. Chest radiography disclosed calcified nodular opacities.

Bronchoalveolar lavage fluid examination showed 120 x 10^6 cells/l, comprising macrophages 56% with siderophages 30%, lymphocytes 9%, polymorphonuclear neutrophils 3%, and eosinophils 4%. Bronchoalveolar lavage fluid examination was normal. Fibre optic bronchoscopy was normal. Bronchoalveolar lavage fluid examination showed 120 x 10^6 cells/l comprising macrophages 56% with siderophages 30%, lymphocytes 39%, polymorphonuclear neutrophils 1%, and polymorphonuclear cells 4%. Bronchoalveolar lavage fluid examination was normal.

Histological study of the temporal artery disclosed a granulomatous inflammation of the vessel wall containing mononuclear cells and histiocytes without giant cells. A search for antinuclear antibodies and cryoglobulin was negative. Histological study of the temporal artery disclosed a granulomatous inflammation of the vessel wall containing mononuclear cells and histiocytes without giant cells.

DISCUSSION

This patient had four of five American College of Rheumatology criteria for the classification of TA, which have a sensitivity of 93.5% and a specificity of 91.2%.

Chronic dry cough without any chest radiograph anomaly is a classical symptom of TA. Machado in Minnesota reported respiratory symptoms in up to 25% of cases of TA.

Cough may be the sole manifestation of the disease for a long time—up to one year. Three year cough course of cough associated with upper limb arteritis was also reported as an initial symptom of TA.

The origin of the cough is not clear. In rare cases, cough might have been attributed to pleural effusion, interstitial pneumonitis, pulmonary vasculitis, or hyperreactive airways.

Blockmans et al found TA lymphocytic alveolitis in three patients with histologically proved TA. Only one patient complained of dry cough and dyspnoea. Chest radiographs and lung function tests were always normal. Cell count was normal on bronchoalveolar lavage fluid examination, but the number of lymphocytes was increased, as in our case, with a percentage varying between 16 and 61%. Discovery of nodular parenchymal densities raises the question of a borderline systemic vasculitis with Wegener granulomatosis.

In our case, there was no obvious cause for intra-alveolar haemorrhage besides TA. Congestive cardiac failure and exogenous agents were excluded. Bacteriological, viral, fungal, and parasitic studies of bronchoalveolar lavage fluid were negative. There was no manifestation suggesting another systemic disease such as microscopic polyangiitis, Wegener's granulomatosis or vasculitis secondary to systemic lupus erythematosus. The favourable outcome with limited dose prednisone in the absence of any other immunosuppressive agent also suggests TA. Cytomegalovirus infection was ruled out, but not other viral infections. Several viruses such as cytomegalovirus, coxsackievirus, adenovirus, enterovirus, mumps, and hepatitis C virus can cause pulmonary haemorrhage. Some authors have suggested that TA has a viral cause, and various agents were incriminated. Varicella zoster and parvovirus B19 DNA were found in temporal biopsy specimens of the elastic lamina.

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Blockmans et al found TA lymphocytic alveolitis in three patients with histologically proved TA. Only one patient complained of dry cough and dyspnoea. Chest radiographs and lung function tests were always normal. Cell count was normal on bronchoalveolar lavage fluid examination, but the number of lymphocytes was increased, as in our case, with a percentage varying between 16 and 61%. Discovery of nodular parenchymal densities raises the question of a borderline systemic vasculitis with Wegener granulomatosis.

In our case, there was no obvious cause for intra-alveolar haemorrhage besides TA. Congestive cardiac failure and exogenous agents were excluded. Bacteriological, viral, fungal, and parasitic studies of bronchoalveolar lavage fluid were negative. There was no manifestation suggesting another systemic disease such as microscopic polyangiitis, Wegener's granulomatosis or vasculitis secondary to systemic lupus erythematosus. The favourable outcome with limited dose prednisone in the absence of any other immunosuppressive agent also suggests TA. Cytomegalovirus infection was ruled out, but not other viral infections. Several viruses such as cytomegalovirus, coxsackievirus, adenovirus, enterovirus, mumps, and hepatitis C virus can cause pulmonary haemorrhage. Some authors have suggested that TA has a viral cause, and various agents were incriminated. Varicella zoster and parvovirus B19 DNA were found in temporal biopsy specimens of the elastic lamina.
Circulating soluble CD40 ligand in patients with eosinophilic fasciitis

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Recently, some reports showed the increased expression of CD40 ligand in autoimmune diseases. CD40 ligand can be expressed in a soluble form. Soluble CD40 ligand (sCD40L) is present in supernatants of in vitro activated T cells in 15 kDa and 18 kDa forms, and these forms are the products of enzymatic cleavage at a metalloproteinase sensitive site in the membrane proximal region of the extracellular domain of the molecule. Their abnormalities have been demonstrated in various kinds of diseases such as chronic lymphocytic leukaemia or systemic lupus erythematosus. But, there has been no report demonstrating the serum levels of sCD40L in patients with eosinophilic fasciitis (EF). In this study we determined the serum levels of sCD40L in patients with EF, and investigated their clinical significance in this disease, in order to evaluate whether sCD40L might be a useful marker for this disease.

METHODS AND RESULTS

Eleven patients (disease duration 1–8 months) with a classic clinical picture of EF, who had received no treatment, were included in this study. All the patients had a recent history of increased skin induration. Skin biopsies including deep subcutaneous tissue and fascia showed markedly inurated and thickened fascia in all cases. Additionally, the thickened fascia contained accumulation of collagen and intense inflammatory cell infiltrates comprising lymphocytes, macrophages, and eosinophils. Clinical manifestations and laboratory findings of each patient were obtained from the medical records. All the laboratory findings were obtained at the time of serum sampling. We also collected control serum samples from 20 healthy volunteers matched for age and sex. Levels of sCD40L were measured with a specific enzyme linked immunosorbent assay (ELISA) kit (Bender MedSystems, Vienna, Austria), according to the manufacturer’s instructions. Values greater than the mean plus 2SD for normal control subjects were regarded as raised. Additionally, serum IgG was evaluated by a turbidimetric immunoassay as described previously. Statistical analysis was carried out with Student’s t test for the comparison of means, and Fisher’s exact probability test for the analysis of frequency. Values of p < 0.05 were considered significant.

Figure 1 shows the serum sCD40L levels in patients with EF and in the healthy control subjects. The serum sCD40L levels in patients with EF were significantly higher than those in the healthy controls (mean (SD) 0.29 (0.16) ng/ml vs 0.13 (0.08) ng/ml, p < 0.01). When the cut off value was set at 0.29 ng/ml (mean + 2SD for the controls), raised serum sCD40L levels were seen in 5/11 (45%) patients with EF.

Serum sCD40L levels correlated significantly with serum IgG levels (r=0.75, p<0.05). On the other hand, serum sCD40L levels were not significantly correlated with serum gammaglobulin, peripheral cell count of eosinophils, erythrocyte sedimentation rate, or serum levels of aldolase. Additionally, three of the five patients with raised serum
sCD40L levels were examined longitudinally before and after corticosteroid treatment for two months to three years. Their clinical manifestations and laboratory abnormalities improved with treatment. Serum sCD40L levels became normal in all three patients (fig 2).

DISCUSSION

As described above, expression of sCD40L in patients with several connective tissue diseases has already been evaluated and shown to be increased. Berner et al reported that increased expression of CD40 ligand on CD4+ T cells in rheumatoid arthritis indicated prolonged and increased activation of CD4+ T lymphocytes and was associated with active disease and, possibly, an unfavourable prognosis.1 Valenti et al found that expression of CD40 ligand in activated CD4+ T lymphocytes was increased in patients with systemic sclerosis.1 On the other hand, Vakkalanka et al reported that the mean concentration of serum sCD40L was statistically significantly higher in patients with systemic lupus erythematosus (SLE) than in disease controls or healthy subjects, and segregation of patients with SLE by severe, moderate, or mild extent of disease showed a relationship between disease severity and sCD40L concentration.1 These findings suggest that CD40 ligand or sCD40L are associated with clinical features of these diseases. In our study, serum levels of sCD40L in patients with EF were significantly higher than those in healthy controls. sCD40L is present in supernatants of in vitro activated T cells.1 In patients with EF, activated T cells were thought to be increased because of the presence of raised interleukin 5 and interleukin 10.1 Thus, our results may reflect such a condition. Additionally, serum levels of sCD40L correlated significantly with serum IgG levels in patients with EF, and serum sCD40L levels normalised after treatment in patients with raised sCD40L levels. As well as peripheral cell counts of eosinophils, erythrocyte sedimentation rate or serum levels of aldolase, hypergammaglobulinaemia is reported to be one of the markers of disease activity, which occurs in 75% of patients and is usually due to a polyclonal increase in IgG.1 Thus, our results suggest that sCD40L is a good marker of EF, reflecting the effects of treatment. However, only 5/11 patients showed raised levels of sCD40L. Thus, the usefulness of sCD40L as a marker of disease activity was not completely substantiated in this study. Additionally, serum sCD40L levels did not correlate with gammaglobulin despite significant correlation with IgG, possibly owing to the small number of patients studied. Moreover, we performed the longitudinal study in only three patients with raised sCD40L levels because other serum samples were not available, so the longitudinal data may be incomplete. Additionally, there is a possibility that corticosteroid treatment, independently of disease, can reduce sCD40L levels. Further studies are needed to clarify the significance of the role of sCD40L in this disease.

REFERENCES