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

![Figure 1](http://ard.bmj.com/)

**Figure 1** Modulation of IL16 transcripts in RA SF. SF of a patient with RA were expanded in culture and analysed for IL16 transcripts during the second passage. The synovial fibroblasts were incubated with phorbol ester (PMA), ionomycin (Iono), cAMP, okadaic acid (Oka), MAS-7, H-7, or staurosporine (Stauro). Untreated cells served as controls (C). RA SF transcribed IL16 encoding mRNA [C]. Staurosporine and MAS-7 enhanced the RT-PCR signal to some extent, whereas in PMA, Oka, and H-7 treated cells the IL16 RT-PCR signal was reduced. A recombinant IL16 plasmid served as a positive control (+) and the RT-PCR master mix without cDNA served as a negative control (−), top. GAPDH RT-PCR served as control to show sufficient quality and quantity of the cDNA employed (bottom). A 100 bp DNA marker shows PCR product sizes (M).

![Figure 2](http://ard.bmj.com/)

**Figure 2** Regulation of IL16 transcripts in OA SF. SF of a patient with OA were expanded and analysed for IL16 transcripts in the second passage. The synovial fibroblasts were incubated with phorbol ester (PMA), ionomycin (Iono), cAMP, okadaic acid (Oka), MAS-7, H-7, or staurosporine (Stauro). Untreated cells served as controls (C). OA SF transcribed IL16 encoding mRNA [C]. cAMP and staurosporine enhanced the RT-PCR signal. In PMA, Iono, Oka, and H-7 treated cells the IL16 RT-PCR signal was reduced. A recombinant IL16 plasmid served as a positive control (+) and the RT-PCR master mix without cDNA served as a negative control (−), top. GAPDH RT-PCR served as control to show sufficient quality and quantity of the cDNA employed (bottom). A 100 bp DNA marker shows PCR product sizes (M).
acid or H-7 dihydrochloride reduced the IL16 RT-PCR signals. As okadaic acid and H-7 reduced the cell viability prominently, the decreased IL16 signals probably result from the induction of cell death. In contrast, incubation of the cells with PMA, ionomycin, cAMP, MAS-7, or staurosporine did not reduce the viability.

Protein kinase inhibitor staurosporine has been reported to induce apoptosis in some cells. Enumeration of dead cells and observation of morphological changes by microscopy upon staurosporine treatment did not give any indication of reduced cell viability at concentrations 10- to 100-fold above the concentrations used in our experiments. RA SF are resistant to induction apoptosis by overexpression of sentrin, Bcl-2, and mutant forms of p53. Therefore the RA SF, especially, may be able to respond to a staurosporine induced pathway with enhanced IL16 transcript amounts. Because protein kinase C activator PMA reduced IL16 transcripts in SF, the data suggest that in SF the transcription of IL16 might be regulated through protein kinase C dependent pathways.

ACKNOWLEDGEMENTS

We thank A Hack for excellent technical service. This study was supported by grants to WKA (fortune 411, in part by DFG Al 16/10–1) and by institutional funding.

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REFERENCES


Obstructive sleep apnoea as a cause of fatigue in ankylosing spondylitis

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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 Epworth 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 (HADS) (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 EdanTec 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

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Table 1 Results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total group n=17 (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>47.1 (12.8)</td>
<td>46.9 (13.5)</td>
<td>0.76</td>
<td>49.0 (7.1) 54</td>
</tr>
<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>
</tr>
<tr>
<td>BASDAI</td>
<td>4.9 (1.8)</td>
<td>4.8 (1.9)</td>
<td>0.59</td>
<td>5.5 (1.3) 4.6 6.4</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 7.5</td>
</tr>
<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</td>
</tr>
<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>7.3 (9.1)</td>
<td>0.01</td>
<td>0.0 (0.0) 0 0</td>
</tr>
<tr>
<td>Body mass index</td>
<td>32.7 (3.1)</td>
<td>32.5 (4.6)</td>
<td>0.13</td>
<td>32.7 (3.1) 30.5 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>Schöbers (cm)</td>
<td>4.8 (4.0)</td>
<td>4.1 (4.2)</td>
<td>0.23</td>
<td>3.0 (1.4) 4</td>
</tr>
<tr>
<td>Chest expansion (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.

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 32.7 (3.1) 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 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 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.

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REFERENCES

Thoracic high resolution computed tomography in patients with ankylosing spondylitis and without respiratory symptoms

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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 radiographies 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 HCRT 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>
<thead>
<tr>
<th>Table 1</th>
<th>Results of chest HRCT in 44 patients with ankylosing spondylitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>20 (45)</td>
</tr>
<tr>
<td>Emphysema</td>
<td>5 (11)</td>
</tr>
<tr>
<td>Interstitial lung disease</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Upper lobe fibrosis</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Ground glass attenuation</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Non-specific interstitial change</td>
<td>20 (45)</td>
</tr>
<tr>
<td>Pleural thickening</td>
<td>12 (27)</td>
</tr>
<tr>
<td>Parenchymal bands</td>
<td>10 (23)</td>
</tr>
<tr>
<td>Subpleural bands</td>
<td>6 (14)</td>
</tr>
<tr>
<td>Blebs</td>
<td>6 (14)</td>
</tr>
<tr>
<td>Parenchymal micronodules</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Irregular interfaces</td>
<td>3 (7)</td>
</tr>
</tbody>
</table>

Chest HRCT of a 37 year old patient showing ground glass aspect (black arrow) with parenchymal band (white arrow).
acknowledgement
Supported in part by a grant from Pfizer.

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References
which typically are the metatarsals or distal tibia as reported by Wijnands and Burgers. These skeletal sites have a high cortical bone content and are under different and potentially greater mechanical strain than the spine or hip site. Thus whether methotrexate causes regional bone loss and whether mechanical strain is important in the pathogenesis of these stress fractures remains uncertain. Other limitations of our study include the relatively small sample size and short duration of follow up (21 months) which may result in type 2 errors. We also had to rely on the Lunar DPX manufacturer’s control database to act as a control group as we did not have an aged match control group at baseline. Other confounding factors are that as the longitudinal phase was some time after initiation of methotrexate, early bone loss might have been missed, although this seems unlikely, as baseline Z scores were normal. We were only able to recruit 20 of the original 30 patients who participated in the cross-sectional phase of this study, but this was owing to patient preference rather than side effects or lack of efficacy of the treatment. All the patients received methotrexate continuously during follow up. Although the dose of methotrexate in the patients studied was relatively low (median weekly dose 9.8 mg), we did not find a relationship between weekly or cumulative dose and bone turnover or BMD (both baseline and longitudinally).

In summary, our findings suggest that weekly methotrexate treatment in the doses used in this study, is unlikely to increase fracture risk at the common skeletal sites for osteoporotic fractures.

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REFERENCES

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 one joint was affected by pain or stiffness, but negative if the consultant’s assessment.

The questionnaire (box 1) to all patients before the consultant and then passed to the nurse practitioner who referred to one consultant for early assessment over a 10 week 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

Box 1 Questionnaire.

- The presence or absence of the following items was recorded.
- Early morning stiffness >1 hour
- Characteristic distribution for IJD
- First degree relative with IJD
- Clinical evidence of synovitis
- ESR ≥20 mm/1st h (men), ≥30 mm/1st h (women)
- Positive rheumatoid factor (≥1/80)
- Erosions on hands or feet x ray
- Benefit from NSAID or steroids

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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. Increased levels of antibodies against ANXV (aANXV) have been reported in patients with different systemic autoimmune disorders as well as in women with recurrent fetal loss and pre-eclampsia. 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 aPL were negative. One 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 ischemic attack (TIA), 25 with ischemic 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 immunosorbent assay (ELISA) for the presence of aANXV, anticardiolipin, anti-β₂-glycoprotein, and anti-prothrombin antibodies. 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, leaving sufficient space for the formation of coagulation complexes. aANXV were shown to induce the apoptosis of endothelial cells, creating a procoagulant environment 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. 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.

REFERENCES


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Accepted 20 June 2002

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Accepted 20 June 2002
CASE REPORT

A woman born in 1926 presented in 1999 with persistent dry cough. She complained of head-ache without fever, jaw claudication, scalp tenderness, and visual or musculoskeletal manifestations. She denied any other upper airways symptoms. Physical examination was normal. Arterial pressure was 140/70 mm Hg. Leucocyte count was 6.8 × 10^9/l with 4.2 × 10^9/l polynuclear neutrophils, 0.2 × 10^9/l eosinophils, haemoglobin 130 g/l, and platelets 9 × 10^11/l. A dipstick urinary test showed no proteinuria and 7 mg daily prednisone. C reactive protein was 14 mg/l. She had a history of pulmonary tuberculosis treated in 1951 with streptomycin and para-aminosalicylic acid. She complained of dry cough and dyspnoea. Chest radiographs and lung function tests were always normal. Cell count was normal on bronchoalveolar lavage fluid examination. The favourable outcome with medium dose prednisone in the authors' previously reported patients with histologically proved TA.

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. Macko 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. A three year 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 T4 lymphocytic alveolitis in three patients with histologically proved TA. Only one patient complained of dry cough and dyspnca. 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 medium 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
patients with histologically proved TA. IgM directed against human para-influenza type 1 virus were associated with TA onset. 3,4 In conclusion, intra-alveolar haemorrhage may be one of the various causes of cough in TA. Further studies are necessary to ascertain whether it is the expression of a primary vasculitis or the consequence of an as yet unknown viral infection.

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Accepted 20 June 2002

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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. 5 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 inured 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 v 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 had IgG, IgA and IgM directed against human para-influenza type 1 virus and human para-influenza type 3 virus. IgM directed against human para-influenza type 1 virus were associated with TA onset.

Circulating soluble CD40 ligand in patients with eosinophilic fasciitis

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FIGURE 1 Serum soluble CD40 ligand (sCD40L) levels determined with specific ELISAs in serum samples from patients with EF and healthy control subjects. Bars show means. The horizontal dotted line represents 2SD above the mean value of healthy controls. p Values were determined by Student's t test.

FIGURE 2 Longitudinal study of serum soluble CD40 ligand (sCD40L) levels in patients with EF.

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. 3 Valentini et al found that expression of CD40 ligand in activated CD4+ T lymphocytes was increased in patients with systemic sclerosis. 3 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. 4 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.

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Intra-alveolar haemorrhage in temporal arteritis

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Ann Rheum Dis 2003 62: 189-190
doi: 10.1136/ard.62.2.189

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