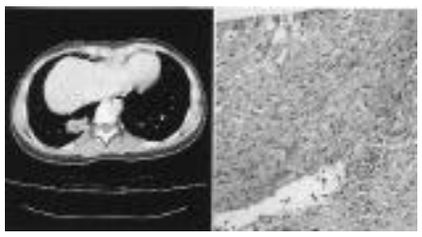

Fig. 1. (**Left**) Contrast-enhanced thoracic CT scan showing a 3 cm diameter mass with a low-density central area in the right posterior basal segment, adjacent to the parietal pleura. (**Right**) Lung biopsy specimen showing an organized thrombus with prominent endovascular recanalisation associated with a granulomatous process within the arterial wall, including multinucleated giant cells, mononuclear infiltration and local periarteritis (hematoxylin-eosin, 20x).

cephalic trunk and total occlusion of the left common carotid and left subclavian artery, with collaterals from the left suprascapular and intercostal arteries. Pulmonary artery pressure was normal on echocardiogram. A consolidation found on chest X-ray was confirmed on thoracic CT scan as a 3 cm diameter mass in the right posterior basal segment (Fig. 1). A needle biopsy showed an organized thrombus with prominent endovascular recanalisation and a granulomatous process within the arterial wall (Fig. 1). The diagnosis of TA with pulmonary parenchymal involvement in the pulseless phase was made (2, 3, 5).

The patient underwent surgical revascularisation with by-pass and was started on prednisone (1 mg/kg), methotrexate (10 mg/m^2/week)and low-dose aspirin. In January 2002 the pulmonary lesion was reduced on CT; at present she continues therapy with methotrexate and aspirin and remains asymptomatic with no signs of active disease (6).

A few cases of TA associated with SpA have been described previously; in these cases both sexes were affected, ages ranged from 15 to 55 years, most patients were HLA-B27 positive; inflammatory bowel disease was reported in two cases (7,8),and the diagnosis of SpA always preceded that of TA. The few reported cases are insufficient to establish a clear link between TA and SpA but they point to common etiological factor(s) and pathogenetic mechanisms (i.e. molecular mimicry based on antigenic homologies between the aorta and bone entheses (9, 10)). Also, aortitis or aortic valve disease in SpA and articular manifestations in TA may be an expression of the link between the two disease.

The only case of SpA associated with TA and pulmonary involvement (1) was similar to our patient in terms of age, sex and the diagnosis of SpA preceeding that of TA. In that case, pulmonary involvement manifested as pleuritic chest pain, fever, and pulmonary infarction documented by chest X-ray and ventilation/perfusion lung scan; in our patient it manifested as exertional dyspnoea and coin lesion on CT scan (Fig. 1). The biopsy of the lesion - not performed in the first case (1) showed histopathological features (Fig. 1) identical to those described as type B lesion by Lie (3) in his series of patients with isolated pulmonary TA. Organized thrombus with prominent recanalization and neoangiogenesis is specific for pulmonary TA, allowing histopathologic differentiation from other lesions such as those found in pulmonary hypertension, granulomatous diseases or vasculitides (3).

In conclusion,our case provides further evidence of the association of TA with SpA. This is the second report of such an association with pulmonary vasculitis and in our case the diagnosis of parenchymal involvement is supported by histopathological findings.

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Molecular cloning and sequence analysis of variant TNF-α transcripts do not indicate a significant role of gene polymorphism in collagen-induced arthritis

Sirs,

In rheumatoid arthritis (RA) tumor necrosis factor alpha (TNF-α) mediates significant proinflammatory events (1). The TNF-α gene is characterized by a variety of polymorphisms but these variations were rarely correlated with RA severity (2-4) The exact biological function and significance therefore remains unclear (5). We wished to study these variations in collagen-induced arthritis (CIA) because inbred animals models greatly facilitate genetic experimentation.

RNA was obtained with a standard Trizol procedure from Dark Agouti rats with severe CIA (n = 8) from previous experiments (6). Investigated tissues included synovium form arthritis. normal joints and healthy spleens. cDNA synthesis was performed using 600 ng DNase treated RNA (Boehringer Mannheim, Germany) with a superscript reverse transcriptase kit (Life Technologies, France). PCR for the rat TNF-α propeptide was adapted from a previously described protocol (7). PCR products were isolated from an agarose gel, purified by electrolution and ligated into a pGEM-T vector (Promega, France). Colonies were screened by TNF-α PCR and cloned. DNA sequencing was performed with a conventional two-directional thermosequenase reaction (Promega) and bidirectional automated sequencing analyses (MWG Biotech, Germany). The sequences were registered at the NIH gene bank. To confirm the presence of intact RNA in inflamed tissue, ribonuclease protection assays (RPA) were performed as previously described (8).

RT-PCR yielded a fragment of 700 bp similar to previous reports (7). DNA sequencing confirmed two distinct cloned inserts (TNF3 and TNF5, NIH accession number AF269160 and AF269159). The 705 bp sized fragment and the open reading frame

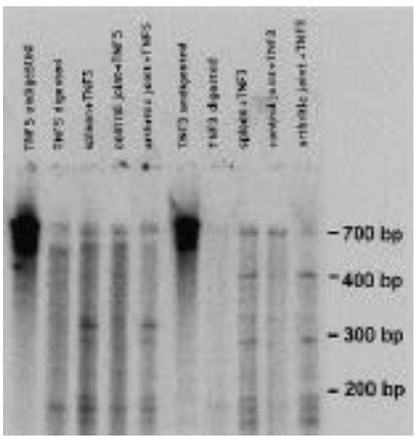

Fig. 1. RPA of TNF-α clones. *In vitro* transcription of TNF3 and TNF5 was performed with [^{32}P]CTP to obtain anti-sense probes. 20 µg RNA from spleen, healthy joints and arthritic joints was hybridised to the anti-sense probes from both TNF-α clones and digested with 40 pg/ml RNase A and 700 units/ml RNase T1. The purified RNA-RNA duplexes were analysed on an urea/acrylamide gel and subjected to autoradiography. Anti-sense probes hybridise to RNA from all tissues. Smaller bands indicate the presence of TNF-α variants with bp mismatches.

corresponded to previous results from rat TNF-α, excluding the stop codon that was not part of the PCR primer (7). The two clones differed at bp 365 (cytosine in TNF3 and thymidine in TNF5) and 568 (adenine in TNF3 and guanine in TNF5). Comparing TNF3 to the NIH database we found two cDNA and 3 DNA sequences with 12, in part repetitive, mismatches (NM_012675, AJ002278, D00475, L00981 and L19123). Eight nt changes (66.6%) were found at the third codon position without amino acid exchanges. Four nt replacements at the first and second codon positions resulted in amino acid exchanges such as proline versus leucine (bp 116 and 365), isoleucine versus threonine (bp 488), lysine versus glutamic acid (bp 566) and phenylalanine versus serine (bp 605). Both TNF-α transcripts were expressed in inflamed and control tissues as indicated by 705 bp RNA protected bands (Fig. 1) suggesting that the two clones are not selectively upregulated in arthritis. Furthermore, nucleotide variations present in arthritic joint-derived cDNA did not result from an error of the PCR-Taq polymerase. The RNase A and TI enzymes used to digest unhybridized RNA are capable of recognizing any single bp mismatches within the RNA-RNA hybrids. Smaller RPA bands indicate the presence of several minor variant sequences without a significant predominance for arthritic tissue.

Based on these results and the analysis from the NIH database, gene polymorphisms do not seem to be of biological relevance because most coding nt variations in the rat species occur without consecutive aa exchanges. Although some singular nt variations cause aa replacements, these changes are unlikely to result in relevant altered peptide confirmations. The described TNF-α polymorphisms thus appear to play a subordinate role in CIA.

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Pancytopenia in ANCA associated vasculitis: Response to immunosuppressive therapy

Sirs,

We report two patients with antineutrophil cytoplasmic antibody (ANCA) associated disease, where severe pancytopenia was a disease manifestation that was improved by immunosuppressive therapy. Microscopic polyangiitis (MPA) is differentiated from polyarteritis nodosa (PAN) by small vessel involvement and the absence of microaneurysms (1). Rapidly progressive glomerulonephritis and pulmonary alveolar haemorrhage are often part of the disease and vasculitic lesions were found in a wide variety of organs (2). Wegener's granulomatosis is a small vessel vasculitis with upper and lower respiratory tract disease, and can have fatal progression in its generalised form (3).

A 37-year-old Caucasian male presented with anterior uveitis, ankle arthritis, recurrent mouth ulcers, nodular vasculitic cutaneous lesions, thrombophlebitis, peripheral sensory neuropathy and intermittent pulmonary shadowing with right pleural pain. Renal biopsy showed diffuse mesangial proliferative glomerulonephritis with no vasculitis or crescents and with no immune deposition. IgG pANCA were positive as were anti-myeloperoxidase antibodies. Antinuclear antibodies were negative. Coeliac axis and mesenteric angiography was normal. A variety of therapies were used starting with prednisolone, followed by intravenous and then oral cyclophosphamide, azathioprine, cyclosporin, thalidomide and methotrexate, over four years. He continued to have flares with cutaneous leucocytoclastic vasculitis lesions (immunofluorescence negative).

He then worsened clinically and presented with severe pancytopenia; haemoglobin (Hb) 6g/dl,platelet (Plt) 50,000/mm^3, white cell count (Wcc) less than 2,000/mm^3, neutrophils (Neu) 500/mm^3 and a MCV greater than 120 Femto/L. Bone marrow biopsy showed all cell lines present and maturing fully. Haematopoetic cells were numerous and adipocytes were sparse. Myelodysplastic changes, probably related to chemotherapy, were also present and included architectural derangement and an increase in immature precursors. On oral cyclophosphamide (100 mgs od) he gradually improved within four months with a Hb 7.3 g/dl,Wcc 4,500/mm^3 and Plt 153,000/mm^3. He is clinically stable on methotrexate (20 mg/week) but still has chronic anaemia, with normal reticulocyte numbers.

The second patient, a 60-year-old Caucasian male, presented with a flare of his inflammatory bowel disease, migratory superficial thrombophlebitis, recurrent non-