VEGF in idiopathic ILD

Simler et al raise an interesting possibility of the prognostic value of plasma VEGF in interstitial lung disease.1 Meyer et al2 in a previous study did not find any difference in serum VEGF levels in patients with diffuse parenchymal lung disease. It would have been interesting to know the bronchoalveolar lavage (BAL) fluid levels of VEGF in these patients as Meyer et al and Koyama et al3 have shown reduced BAL fluid VEGF levels in interstitial lung disease. This might simply reflect damage to the alveolar epithelium (a known major source) in this disease or, indeed, VEGF may have an important role in the pathogenesis of interstitial disease. Interestingly, VEGF receptor blockade has been shown to lead to an induction of apoptosis and an emphysema-like histological appearance in rats but with no evidence of fibrosis or inflammatory cells.4

In addition, it is interesting to speculate on the cellular source of the increased plasma levels of VEGF in the more fibrotic patients. Control of the alveolar-capillary membrane damage with leakage of intra-alveolar VEGF which is known to be compartmentalised and hence lower BAL fluid levels as described in the previous studies? Or does it represent an inflammatory cell source of systemic VEGF correlating with an inflammatory response that is here associated with a poorer outcome? Or is there some other mechanism?

Finally, Koyama et al have shown that smokers also have reduced BAL fluid levels of VEGF and this may be of relevance (if it is close to or part of the fit-1 receptor binding site and the other antibody B reacts with an epitope well removed from this site, with an identical absolute amount of VEGF in the plasma sample, antibody A would read low or negative and antibody B would read high in relation to the amount of sflt-1 present in the plasma sample. We have in fact shown that the capture antibody used in the Quantikine VEGF ELISA is, indeed, sensitive to the presence of sflt-1.6

In addition to this potential variation in the level of free VEGF and VEGF-sflt-1 complexes further confounding species is the amount of placenta growth factor (PLGF). VEGF is a natural homodimer but it does form heterodimers with PLGF and we have detected such complexes.7 Antibodies detecting epitopes that are variably modulated by the binding of PLGF to VEGF will read low or high depending on the PLGF concentration. This focuses on the nature of the R&D Systems monoclonal antibodies to VEGF—one in the Quantikine kit and the other in the Duoset combo. Following the recognition of a difference in performance of these two assays, we contacted R&D Systems for information concerning the nature of these antibodies. We were interested to know if the same antibody or different antibodies were used and what information was available on their specificity. The response from R&D Systems was that the capture antibodies were different, so the concept outlined above is a possible explanation.

It might simply reflect the difficulties in interpreting absolute levels of VEGF in complex media such as plasma. To do this rigorously one ought to quantitate not only free VEGF but also VEGF complexes with sflt-1, sKDR, and PLGF and understand how these unassembled species coexist or compete against each other, and currently this is not possible. It would be simplistic to think that the Quantikine kit values are the “true” VEGF values and the Duoset assay values arfactual. It might suggest that VEGF complexes are free VEGF and the Duoset values total VEGF (free VEGF plus VEGF complexes).

References

Authors’ reply
Dr Medford laudably highlights the interesting findings of other authors regarding levels of vascular endothelial growth factor (VEGF) in idiopathic interstitial pneumonia (IIP). Indeed, our data reflect the findings of Meyer et al who studied 11 patients with IIP.3 We extend their observations in a larger cohort of patients (n = 49) and specifically relate plasma VEGF levels to disease progression and extent of fibrosis on HRCT scanning.2 Indeed, HRCT scanning is perhaps the most reliable surrogate for the extent of disease.6 Like Meyer et al, we observed reduced levels of bronchoalveolar lavage (BAL) fluid levels of VEGF in patients with IIP (91 pg/ml) compared with controls (204 pg/ml). The reduction in the BAL fluid level of VEGF may reflect the absence of angiogenesis in that specific part of the lung, with the plasma VEGF level identifying a secondary phenomenon of compensatory angiogenesis in alternative areas of the lung. Alternatively, VEGF levels appear to be higher in epithelial surface fluid than in the serum, suggesting vectorial intraluminal secretion and the existence of a concentration gradient from air spaces to intravascular spaces.

Thickett et al are correct in their quotation of the normal range for VEGF in plasma (36–76 pg/ml) as measured by the R&D Systems Quantikine ELISA kit.7 They point out that this range is quoted by the kit manufacturers to know if the same antibody or different antibodies were used and what information was available on their specificity. The response from R&D Systems was that the capture antibodies were different, so the concept outlined above is a possible explanation.

It might simply reflect the difficulties in interpreting absolute levels of VEGF in complex media such as plasma. To do this rigorously one ought to quantitate not only free VEGF but also VEGF complexes with sflt-1, sKDR, and PLGF and understand how these unassembled species coexist or compete against each other, and currently this is not possible. It would be simplistic to think that the Quantikine kit values are the “true” VEGF values and the Duoset assay values are artefactual. It might suggest that VEGF complexes are free VEGF and the Duoset values total VEGF (free VEGF plus VEGF complexes).
CARD 15 gene mutations in sarcoidosis

In the last few years substantial progress has been made in unravelling the genetic basis of susceptibility to Crohn’s disease. Three CARD 15 (previously called NOB1) variants, resulting in proteins with modified carboxy terminal regions, have been implicated. 43% of patients with Crohn’s disease carry at least one of these CARD 15 mutations (compared with 15% of healthy controls). Mutations in CARD 15 have also been identified in affected members of families with Blau syndrome. This is a rare autosomal dominant disorder, sometimes referred to as familial sarcoidosis, characterised by granuloma formation in joints, skin, and uvea. CARD 15 is a microbial sensing protein involved in innate immunity. It recognises conserved structural components of microorganisms (bacterial muramyl dipeptide, MDP and peptidoglycan, PGN) and is part of the danger signal pattern recognition network which forms the front line of protective immunity. Mutations associated with Crohn’s disease render the molecule insensitive to MDP and interfere with the downstream activation of NF-kB. One potential result of this may be the persistence of inflammation resulting in engagement of other arms of the adaptive immune system and formation of granulomas. Expression of CARD 15 in monocytes (precursors of macrophages and granulomas) further supports a role for CARD 15 in granuloma formation. We hypothesised that mutations in CARD 15 may be a unifying defect in the multisystemic granulomatous diseases of Crohn’s disease, sarcoidosis, and Blau syndrome.

To investigate this we recruited a cohort of 29 patients with sarcoidosis from the Oxford Centre for Respiratory Medicine. All had a typical clinical picture of sarcoidosis and either histologically proven disease or characteristic Lojgren’s syndrome (defined as an acute onset of disease with erythema nodosum, joint pains, and bilateral hilar lymphadenopathy). The diagnosis of sarcoidosis was also supported by the characteristic appearance of the lungs on a high resolution computed tomographic (CT) scan in all patients. The definition and diagnosis of sarcoidosis adheres to the statement on sarcoidosis adopted by the National Joint Committees of WASOG/ATS/ERS. The patients were first diagnosed between the ages of 25 and 40 years and had been followed up for at least 1 year before recruitment. All were white and one third presented with Lojgren’s syndrome. Written informed consent was obtained for genetic analysis and the study was approved by the local ethics committee.

The entire coding region of CARD 15 (11 exons and flanking intronic sequences) was screened for the presence of mutations. In brief, the CARD 15 gene was amplified from the genomic DNA samples by polymerase chain reaction (PCR) using primers as previously described and sequenced on an ABI 377 automated sequencer using a Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer Applied Biosystems, CA, USA). Sequence data were then aligned using the Sequence Navigator analysis software Version 1.0.1 (Perkin Elmer Applied Biosystems) and compared with the known CARD 15 sequence (EMBL accession number AJ303140).

435 sequence analyses were performed in the 29 patients. We were therefore able to detect new alleles putatively associated with the sarcoidosis phenotype with frequencies as low as 2%. The three mutations associated with Crohn’s disease were specifically examined. The R702W mutation was observed in one patient with sarcoidosis while the G908R and the 1007fs mutations were not found. These results were not different from those reported previously in control populations where the mutations R702W, G908R and 1007fs were present in 4%, 1%, and 2% of 103 European healthy controls. Further, although we did not detect any genetic variation in our sarcoidosis patients. No additional mutations were seen within the rest of the coding region of the gene, suggesting that there were no specific alternative CARD 15 mutations associated with sarcoidosis. Schuurman and colleagues recently found no correlation between specific CARD 15 polymorphic alleles and patients with sarcoidosis from families with more than one member afflicted by the disease. This study provides evidence that CARD 15 is not associated with non-familial sarcoidosis (in patients with a white ethnic background) and that there are no mutations in any part of the coding region of the CARD 15 gene in the patients with the sarcoidosis phenotype.

There is little doubt that there is a genetic predisposition in sarcoidosis, as indicated by the presence of familial clustering, ethnic susceptibility, and recent evidence of an association with HLA-DRB1. Furthermore, for this susceptibility gene(s) has recurrently pointed to a locus near the HLA DR region on chromosome 6. This and the exclusion of the NOB2 locus has focused attention on abnormalities in antigen presentation and cytokine/chemokine receptors as a potential basis for the aetiology of sarcoidosis.

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References
Mycobacterium kansasii is the second most commonly encountered pulmonary mycobacteria associated with lung disease in the United States. Diseases are almost always cavitary, involving the upper lobes with fibrosis and granulomatous inflammation. To our knowledge, M. kansasii is the second most common cause of pulmonary disease, including pulmonary tuberculosis and M. avium-intracellulare infection. Nocardia asteroides infection has never been reported.

Malformations characterised by the presence of normal lung parenchyma. While the patient had been noted to have asymptomatic congenital lung malformations, the patient had been noted to have asymptomatic congenital lung malformations.

The infection of the sequestration through Kohn's foramen was thought to be responsible for the infection of the sequestration.

Microscopic examination showed no endobronchial abnormality. A chest radiograph taken at admission and a computed tomographic scan showed a patchy consolidation over the left basal lung. Magnetic resonance imaging (MRI) and angiography of the chest showed that the sequestration of the left bronchus had been noted and cystic formation and profuse sputum were found within the lesion. The three feeding arteries from the aorta at the level of the 10th thoracic spine were seen. Microscopically, the sequestration revealed bronchiolitis and microabscess formation. Granulomatous inflammation was also present, but no organisms were identified by acid fast and Grocott's methenamine silver (GMS) stains.

In our case, M. kansasii was the first organism thought to be responsible for the infection of pulmonary sequestration in the absence of other pulmonary or extrapulmonary involvement. It is assumed that the organism reaches the sequestration through Kohn's foramen without causing evident disease in the normal lung parenchyma.

The combination of isoniazid, rifampin, and ethambutol was recommended for treatment of M. kansasii pulmonary disease. However, surgical removal should be advocated in both asymptomatic and symptomatic cases of pulmonary sequestration and the benefit of a period of preoperative antimicrobial treatment in infected pulmonary sequestration has been debated. Our patient simply underwent a surgical resection and did not receive any anti-mycobacterial agents.

We have reported the unique occurrence of pulmonary sequestration with M. kansasii infection. Surgical resection allows establishment of the exact diagnosis and immediate removal of the infectious focus, thus preventing complications related to the infection or to the malformation itself.

Infectected pulmonary sequestration caused by Mycobacterium kansasii

Mycobacterium kansasii is the second most commonly encountered pulmonary mycobacteria associated with lung disease in the United States. Diseases are almost always cavitary, involving the upper lobes with fibrosis and granulomatous inflammation. Underlying pulmonary diseases associated with M. kansasii infection include pneumoconiosis, chronic obstructive lung disease, AIDS and malignancy. To our knowledge, M. kansasii has never been reported in the English literature in the aetiology of infected pulmonary sequestration. We report a case of intralobar sequestration complicated by infection with M. kansasii.

A 33 year old man was previously healthy. An abnormal finding on the chest radiograph had been noted at a routine health check 1.5 years earlier. He had no history of medical illness and no risk factors for HIV infection. No symptoms attributed to his respiratory system, such as cough, haemoptysis or dyspnoea, were noted. Constitutional symptoms including malaise, fever, night sweats, anorexia, or weight loss were lacking. Physical examination revealed systolic bruit at the left basal lung. There was neither cyanosis nor digital clubbing. Oxygen saturation by pulse oximetry was 99% while breathing room air. Blood laboratory studies disclosed a white blood cell (WBC) count of 7.4×10^9/L (neutrophils 60.7%, lymphocytes 13.6%), and the level of carcinoembryonic antigen (CEA) was 1.35 ng/mL (reference value <3 ng/mL). Sputum acid-fast stain and mycobacterial cultures were all negative.

Figure 1 Magnetic resonance imaging (MRI) and angiography of the chest showing the aberrant bronchial artery (arrow) arising from the thoracic aorta crossing the consolidation.

Figure 2 Microscopic section of the lung parenchyma showing microabscesses and granulomatous inflammation (arrow).

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