If you have a burning desire to respond to a paper published in Thorax, why not make use of our "rapid response" option? Log on to our website (www.thoraxjnl.com), find the paper that interests you, and send your response via email by clicking on the "letters" option in the box at the top right hand corner.

Providing it isn't libellous or obscene, it will be posted within seven days. You can retrieve it by clicking on "read letters" on our homepage.

The editors will decide as to whether to publish it in a future paper issue.

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 al. 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 al. 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 encephalomyelitis-like histological appearance in rats but with no evidence of fibrosis or inflammatory cells.2

In addition, it is interesting to speculate on the cellular source of the increased plasma levels of VEGF in the more fibrotic patients. Could there be 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 smoker's also have reduced BAL fluid levels of VEGF and this may be of relevance (if intrapulmonary VEGF is postulated as having a role in this disease), given that the patients with desquamative interstitial pneumonia had all smoked compared with 50% of those with non-specific interstitial pneumonia and only 20% of the controls.

A R L Medford

Lung Research Group, Division of Medicine, Southmead Hospital, Bristol BS8 3LP, UK; andrew.medford@bristol.ac.uk

References


5 Kaner RJ, Brenchley PE, Horrocks AW, et al. Lung Research Group, Division of Medicine, Southmead Hospital, Bristol BS8 3LP, UK; andrew.medford@bristol.ac.uk

Authors' reply

Dr Medford laudably highlights the interesting findings of other authors regarding levels of vascular endothelial growth factor (VEGF) in idiopathic interstitial pneumonitis (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.4 Indeed, HRCT scanning is perhaps the most reliable surrogate for the extent of disease.5 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.6

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 kit.7 They point out that this range is quoted by the kit manufacturers and is consistent with their own data and, indeed, with other published studies.8 We did not use the Quantikine kit in this study but stated clearly that: “The ELISA capture and detection antibodies for assaying IL-8 and VEGF were selected paired reagents optimised for ELISA performance from R&D Systems”. R&D currently sell these paired reagents under the name “Duoset”.

Different ELISA formats for VEGF quantitation using recombinant VEGF165 as standard are available. Capture reagent: (1) rabbit polyclonal anti-VEGF (in house); (2) soluble flt-1 (Sflt-1); (3) Quantikine kit, mouse anti-VEGF; (4) Duoset, mouse anti-VEGF. Detection reagent: (1) mouse anti-VEGF (Genetech 4.6.1); (2) rabbit anti-VEGF; (3) mouse anti-VEGF; (4) mouse anti-VEGF. Not surprisingly, each assay reports a different normal range. In our experience the Quantikine kit measures low (with up to a third of samples having undetectable levels) and the Duoset combination measurements high, as we reported in the article (648 pg/ml). The other assays report intermediate values.

A number of possibilities exist as to why these assays read differently. It is unlikely to be due to platelet release as suggested. The greatest difference in VEGF levels that is detected between paired serum (complete platelet release of VEGF) and standard plasma samples (low platelet VEGF release) is at most only three to four fold. Similarly, the difference in VEGF between paired platelet poor and platelet rich plasma samples is of the same order of magnitude. To demonstrate this one has to spin plasma samples at 2700g for 15 min to prepare platelet poor plasma samples. In our experience it is important to use plasma rather than serum samples to optimise assay performance. We treat each study sample similarly in terms of centrifugation—whether it be 300g for 12 min, 1000g for 15 min, or 2700g for 15 min. This will minimise variation in the study samples due to the “platelet release effect”.

There are possible explanations for the VEGF immunoassays reading differently in plasma. It may be a combination of at least two effects—the nature of the epitope detected and the presence of other competing ligands in the sample. Different antibodies raised to VEGF will react variably with available epitopes on the ligand. This can be quite striking with monoclonal antibodies to different epitopes of a ligand when they are used as ELISA capture reagents.8 Antibody A reacts with an epitope on VEGF that is close to or part of the flt-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-1.9 In addition to this potential variation in the level of free VEGF and VEGF-sflt-1 complexes in plasma samples, a 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.10 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. 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 question outlined above is a possible explanation.

It is indeed 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 then understand how one assay measures against another, 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 artefactual. It might simply reflect the fact that Quantikine values are free VEGF and the Duoset values total VEGF (free VEGF plus VEGF complexes).
The important observation in our study is not the absolute VEGF plasma values but the relative differences in VEGF levels between patients and control samples over time. Where the sampling issues have been fully appreciated and rigorously controlled to allow clinical interpretation of the results. We have emphasised the prognostic value of plasma VEGF in idiopathic pulmonary fibrosis and have shown a significant positive relationship between the HRCT fibrosis score and the plasma concentration of VEGF. A comparison with acute respiratory distress syndrome (ARDS) is not useful and perpetuates the concept that ARDS equates with chronic idiopathic pulmonary fibrosis, which is not the case.

In relation to the point about quantitating local VEGF concentrations in the lung where the influence of epithelial sft-l might be greater, it is to be expected that different assays could lead to a variation in reported VEGF levels for the reasons already discussed.

In conclusion, we have complete confidence in the validity and reproducibility of the VEGF data presented. In a situation of excess VEGF production which could potentially be driving an angiogenic fibrotic process in the lung, we suggest it is entirely appropriate to contemplate antagnoising excess VEGF in order to control the disease pathophysiology. Overdosing on VEGF antagonist is clearly counterproductive and, as stated, could lead to the apoptosis of both epithelial and endothelial cells. Clearly, it is a case of restoring homeostasis.

J Salom
Department of Respiratory Medicine, Mater Misericordiae Hospital, University College Dublin, Dublin 7, Ireland

P E Brenchley
Renal Research Laboratory, Manchester Royal Infirmary, Manchester M3 9WL, UK

N R Simler
Royal Brompton Hospital, London, UK

J J Egan
Department of Respiratory Medicine, Mater Misericordiae Hospital and St Vincents University Hospital, University College Dublin, Dublin 7, Ireland

Correspondence to: Dr J J Egan, Advanced Lung Disease and Lung Transplant Program, Dublin Molecular Medicine Center, The Mater Misericordiae Hospital and St Vincents University Hospital, University College Dublin, Dublin 7, Ireland; jegan@mater.ie

References


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 NOB2) gene variants, resulting in proteins with modified carboxy terminal regions, have been implicated. 14% of patients with Crohn’s disease carry at least one of these CARD 15 mutations (compared with 1% of healthy controls).1 Mutations in CARD 15 have also been identified in affected members of families with Blau syndrome.2 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-xB. One potential result of this may be the persistence of antigen 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 an involvement 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 Löfgren’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 in the European respiratory society.1 This patient was 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 Löfgren’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 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, 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 A303140). 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 the 1007fs were present in 4%, 1%, and 2% of 103 European healthy controls. Furthermore, they did not differ from data derived from healthy controls recruited in the UK.4 The codons 334 and 469 reported to be involved in Blau syndrome were also carefully scrutinised but 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.

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-DR8.7 Further studies for this susceptibility gene(s) has recently 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.

L P Ho, A J McMichael
MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Oxford University, Oxford OX3 9DS, UK

L P Ho, R J O Davies
Oxford Centre for Respiratory Medicine, Churchill Hospital, Oxford OX3 7JU, UK

K Gaber
Royal Devon and Exeter Hospital, Exeter EX2 5DW, UK

J-P Hugot, F Merlin
Fondation Jean Dausset/CEPH, Paris, France

J-P Hugot
INSERM U458, Hospital Robert Debré, AP-HP, Paris, France
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 7.4 ng/ml. Sputum acid-fast stain and mycobacterial cultures were all negative. Bronchoscopic 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 an aberrant bronchial artery had arisen from the left side of the thoracic aorta and crossed the territory of the consolidation patch, but the venous drainage could not be visualised clearly (fig 1). The patient underwent lobectomy of the left lower lobe. Histologically, the postoperative specimen disclosed a well-organized granulomatous reaction characterized by multinucleated giant cells and lymphocytes, similar to those found in sarcoidosis, with no evidence of caseation. The final diagnosis was pulmonary sequestration due to mycobacteria 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.

S H Lin
Department of Internal Medicine, Far Eastern Memorial Hospital, Taipei County, Taiwan

L N Lee, Y L Chang, Y C Lee, L W Ding, P R Hsueh
Departments of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan

Correspondence to: Dr P R Hsueh, Departments of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, No 7, Chung Shan South Road, 100 Taipei, Taiwan; hsporen@ha.mc.ntu.edu.tw

References