Virchows Arch (1998) 432:61–65

ORIGINAL ARTICLE

Edina Tolnay · Cornelius Kuhnen · Bruno Voss
Thorsten Wiethege · Klaus-Michael Müller

Expression and localization of vascular endothelial growth factor and its receptor *flt* in pulmonary sarcoidosis

Received: 22 May 1997 / Accepted: 21 August 1997

Abstract Vascular endothelial growth factor (VEGF) is a multifunctional cytokine, which has recently been reported to enhance the activation and migration of monocytes through the *flt* receptor in vitro, which are key events in granuloma formation of granulomatous disorders and in sarcoidosis. Since activated macrophages and monocytes are known to be involved in sarcoid granuloma formation in sarcoidosis, we investigated the expression of VEGF and its receptor *flt* in 33 paraffin-embedded lung tissue biopsies of patients with pulmonary sarcoidosis. VEGF-mRNA was localized by nonradioactive in situ hybridization, VEGF and *flt* expression were visualized immunohistochemically. We found an increased transcription and protein production of VEGF and an overexpression of *flt* in activated alveolar macrophages, in epitheloid cells, and in multinuclear giant cells of pulmonary sarcoid granulomas.

Key words Vascular endothelial growth factor · *flt* · Pulmonary sarcoidosis · Activated macrophage

Introduction

Sarcoidosis is a multisystem granulomatous disorder of unknown aetiology, which is found worldwide. The diagnosis is usually based on radiographic and clinical findings and supported by histological demonstration of sarcoid granulomas; other granulomatous diseases of known aetiology, tuberculosis, berylliosis or sarcoid-like reaction associated with malignant tumours, should be excluded [20]. Granuloma formation is the characteristic feature of sarcoidosis and is thought to be a host response to an unknown antigen. This substance is not destroyed by an acute inflammatory reaction and provokes mononuclear phagocyte activation [1]. The sarcoid granuloma consists of various forms of activated macrophages (epitheloid cell, multinuclear giant cell) and activated T-lymphocytes at the site of the disease activity. The activation of these cells is mediated by a number of lymphokines and soluble macrophage factors, but T-cells are thought to regulate the recruitment of mononuclear phagocytes predominantly.

Sarcoidosis is often associated with nongranulomatous microangiopathic lesions in various other organs [15]. Increased angiogenesis-inducing ability of activated alveolar macrophages is found in bronchoalveolar lavage of patients with pulmonary sarcoidosis, but the factor responsible has not been identified [14]. Berse et al. reported an increased gene expression of vascular endothelial growth factor (VEGF) in activated alveolar macrophages [3], but no data are available on VEGF expression in these cells or other activated forms of mononuclear phagocytes in sarcoidosis. In addition, it is reported that tumour-associated activated macrophages and activated peritoneal macrophages of patients with endometriosis have the capacity to initiate and enhance angiogenesis overexpressing VEGF [13, 21].

VEGF is a heparin-binding, dimeric gylcoprotein with a selective mitogenic effect on vascular endothelial cells in vitro and a direct angiogenic effect in vivo [6]. Differential splicing of a single gene transcript results in four protein isoforms (121, 165, 189 and 206 amino acids) of the growth factor, with various biological activities. Two high-affinity tyrosine-kinase receptors are identified for VEGF in humans: KDR (or *flk*-1) and *flt*, which are expressed on vascular endothelial cells. Based on experimental data, KDR is thought to be the major regulator of angiogenesis, while *flt* is involved mainly in endothelial organization in embryogenesis. VEGF enhances the migration of monocytes [4] and neutrophil granulocytes [2] and is a chemoattractant for mast cells in the picomolar

E. Tolnay
Department of Pulmonology, Semmelweis Medical School, Budapest, Hungary

C. Kuhnen · T. Wiethege · K.-M. Müller (✉)
Institute of Pathology, University Clinic Bergmannsheil, Bürkle-de-la-Camp Platz 1, D-44789 Bochum, Germany
Tel.: (++49) 234-302 6600, Fax: (++49) 234-302-6671
e-mail: patho-bhl@ruhr-uni-bochum.de

B. Voss
Professional Associations' Research Institute for Occupational Medicine, Bochum, Germany

range [8]. Recently, a possible role of VEGF in the recruitment and activation of monocytes has been reported in vitro, mediated through the *flt* receptor [2, 5].

We investigated 33 cases of pulmonary sarcoidosis, looking for increased transcription and/or production of this potent angiogenic cytokine and its receptor *flt* in activated alveolar macrophages or in other activated forms of mononuclear phagocytes, such as multinuclear giant cells or epitheloid cells, in sarcoid granulomas.

Materials and methods

Formalin-fixed and paraffin-embedded bronchoscopic (30) and thoracoscopic (3) biopsy specimens from the archives of the Institute of Pathology were investigated retrospectively in the present study. Biopsies were obtained by bronchoscopy or by open lung biopsy as part of routine clinical diagnosis in patients with clinically and/or radiologically suspected pulmonary sarcoidosis. There were 10 female and 23 male patients, their median age being 39 years (range: 23–79). Two patients had previously histologically confirmed sarcoidosis, and were rebiopsied because of symptoms suggesting recurrence of the disease. None of the patients was receiving steroid treatment at the time of the diagnosis. The diagnosis of pulmonary sarcoidosis was based on clinical data (symptoms, radiological signs) and histomorphologically confirmed by presence of sarcoid granulomas in haematoxylin-eosin and elastica-van Gieson-stained sections [20].

For comparison archival paraffin-embedded transbronchial biopsy specimens that had no signs of pathological alterations and had also been obtained during diagnostic procedures were investigated.

Specimens were analysed for VEGF expression with a rabbit polyclonal antibody against human VEGF (Santa Cruz, Calif.) which recognizes the 121-, 165- and 189-amino acid splice variants of VEGF. Immunohistochemical staining for the *flt* receptor was carried out using a rabbit polyclonal antibody raised against a carboxy terminal of the human *flt* peptide (Santa Cruz, Calif.). Activated macrophages and multinuclear giant cells were highlighted by a monoclonal mouse anti-CD 68 antibody (DAKO, Hamburg, Germany).

Immunostaining was performed using the alkaline-phosphatase-anti-alkaline phosphatase method (APAAP) [7]. Sections 4 µm thick were cut consecutively from formalin-fixed and paraffin-embedded tissue samples, mounted on poly-L-lysin-coated slides and dried overnight at 58° C. Paraffin sections were dewaxed by xylene, rehydrated with graded concentrations of ethanol and finally washed in Tris-Buffer (pH 7.6) for 10 min. VEGF required proteinase K predigestion in a working solution of 0.4 mg/ml (Dako) for 10 min at room temperature. For optimal retrieval of the *flt* and CD 68 antigen, sections were boiled in citrate buffer (pH 6.0) (Dako) in a microwave oven at 600 W for 5 min. This step was repeated four times. The following steps were finalized by an automated staining system, Dako TechMate 500.

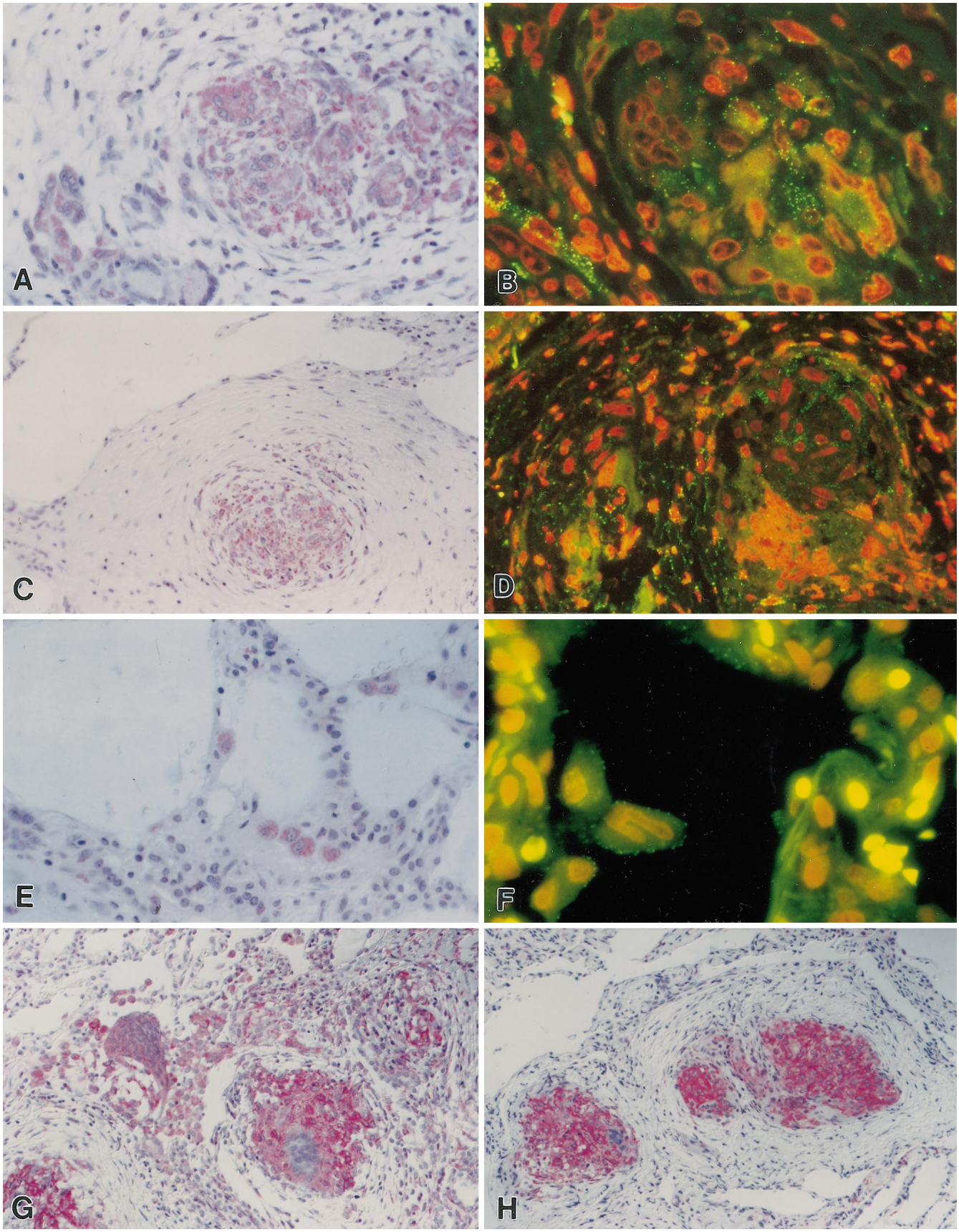
Sections were incubated with the primary antibody solution for 25 min at room temperature, using a working dilution of the antibodies 1:100 for *flt*, 1:200 for CD 68 and 1:400 for VEGF. Slides were rinsed once in buffer (Puffer Kit, Dako). For *flt* and VEGF immunostaining, sections were incubated with the secondary antibody, a mouse anti-rabbit serum, in a 1:150 concentration (Dako) for 25 min at room temperature. Slides were rinsed in buffer (Puffer Kit, Dako). Immunoreaction was demonstrated with the APAAP Kit (Dako) according to the specifications of the manufacturer. The secondary antibody was an alkaline phosphatase-labelled monoclonal calf antibody, and the bridging antibody a monoclonal anti-calf mouse antibody.

Sections were incubated with the chromogene alkaline-phosphatase substrate (Fast Red, DAKO) for 20 min at room temperature. Finally, sections were counterstained by Mayer's haematoxylin (DAKO) for 1 min, dehydrated in graded ethanol and coverslipped. For positive controls fetal kidney tissue was stained for VEGF and normal lymph node tissue, for CD 68. Negative controls used all reagents except the primary antibody.

For nonradioactive in situ hybridization a digoxigenin-labelled cDNA for human VEGF (a generous gift of Professor W. Risau, Max-Planck-Institut für physiologische und klinische Forschung, Bad Nauheim, Germany) was applied. The cDNA probe for human VEGF was a 520-bp fragment of clone VEGF 121 subcloned into the EcoRI/BAM HI site of Bluescript KS [19]. The probes were labelled by digoxigenin using the random primed oligolabelling method with the Dig-Labeling Kit (Boehringer Mannheim, Germany).

We used a slightly modified in situ hybridization protocol described by Wiethege [23]. Paraffin sections 4 µm thick were applied to glass microscope slides, which had previously been immersed in 2% TESPA (3-aminopropyltrietoxysilane, Sigma, Germany) in acetone for 5 s and twice in acetone for 1 min each. Slides were dewaxed in xylene and rehydrated in decreasing ethanol concentrations (99 vol%, 90 vol%, 70 vol%, 50 vol%) in deionized water. After washing in phosphate-buffered saline (PBS, pH 7.4) and dehydration in graded series of ethanol (30 vol%, 70 vol%, 90 vol% and 100 vol%), samples were air-dried and treated by sequential incubation as follows: 0.2 N HCl (20 min), double-distilled water (5 min), 0.125 mg/ml pronase (Serva, Heidelberg, Germany, 10 min), 0.02 M gylcin (Merck, Darmstadt, Germany; 30 s), twice PBS (30 s). Specimens were postfixed in 4% paraformaldehyde/PBS for 20 min and washed in PBS (5 min). After incubation in 0.1 M triethanolamine, pH 8.0, Merck) containing freshly added 0.25 vol% acetic anhydride for 10 min and dehydration in serial alcohols (30 vol%, 70 vol%, 90 vol% and 99 vol%) the sections were air-dried. All steps were performed at room temperature and all solutions were treated with diethylpyrocarbonate (Sigma). For prehybridization, samples were covered with 300 µl of prehybridization buffer solution containing 50% deionized formamide (Fluka, Germany), 0.3 M NaCl, 10 mM Tris pH 7.5, 10 mM $NaHPO_4$ pH 6.8, 5 mM EDTA, 0.1× Denhardt's solution, 10 mM dithiotreitol (Sigma), 0.25 mg/ml yeast tRNA (Sigma), 12.5% dextransulfate (Pharmacia Biotech, Sweden), and 0.5 mg/ml salmon sperm DNA (Sigma) and incubated in a humid chamber for 2 h at 42° C. For hybridization, prehybridization mix was removed and slides were covered with 30 µl of hybridization solution, containing 1 µg digoxigenin-labelled cDNA probe/ml. The slides were coversliped, sealed with rubber cement and incubated in a humid chamber for 18 h at 42° C. After removal of the coverslips, slides were washed twice in 50% formamide/2 ×SSC (standard saline citrate)/1% mercaptoethanol for 20 min at 44° C and in 2×SSC and then 0.1×SSC for 20 min at 51° C. Finally specimens were rinsed for 3 min in PBS and covered with 30 µl of fluorescein-isothiocyanate (Boehringer Mannheim)-conjugated anti-digoxigenin antibody diluted 1:20 in 5% normal sheep serum in PBS. Sections were coverslipped and incubated in a light protected humid cham-

Fig. 1 Immunohistochemical staining for **A**, **C**, **E** vascular endothelial growth factor (VEGF) and **G**, **H** *flt* and **B**, **D**, **F** non-radioactive in situ hybridization of VEGF mRNA in pulmonary sarcoidosis. **A** Multinuclear giants cells and epitheloid cells stained positive with anti-VEGF antibody, while stromal cells and lymphocytes did not react with anti-VEGF antibody. ×400. **B** High signal intensity of VEGF mRNA in multinuclear giant cells and epitheloid cells. ×625. **C** Overview of an early granuloma with homogeneous VEGF staining pattern. No detectable immunostaining in normal alveolar endothelial cells and stromal cells. ×200. **D** Transcripts of VEGF mRNA in an epitheloid granuloma. ×312. **E** Activated alveolar macrophages stained strongly positive for VEGF ×400. **F** VEGF mRNA signals in activated alveolar macrophages. No detectable signals of VEGF transcripts in alveolar endothelial cells or pneumocytes. Granuloma formation and an early granuloma. ×625. **G** *flt* Receptor expressed at a high level in activated alveolar macrophages and in epitheloid cells, but centrally located multinuclear giant cells stained negative with the anti-*flt* antibody. **H** Pulmonary sarcoid granulomas without alveolitis. Epitheloid cells and a multinuclear giant cell showed a positive immunoreaction against anti-***flt*** antibody. ▶


A
B
C
D
E
F
G
H

ber for 2 h at 37° C. After two washes for 10 min in PBS, specimens were counterstained with 30 µl of propidiumiodide in PBS (500 ng/ml; Boehringer Mannheim) for 5 min. After removal of excess propidiumiodide washing with PBS for 10 min, slides were dehydrated by a series of alcohol dilutions (70 vol%, 90 vol%, 99 vol%) and air-dried. Finally, specimens were mounted in a glycerol/PBS solution, Citifluor (Plano, Germany) [11]. As negative controls, sections were hybridized with a digoxigenin-labelled pBR322 DNA probe (Boehringer Mannheim).

We used an epiluminated fluorescence microscope (Leitz, Germany) for the evaluation.

Results

Expression of VEGF was established by the dark purple reaction product obtained by the APAAP technique. Normal bronchial epithelial cells, stromal cells and pneumocytes showed no detectable VEGF immunostaining. Smooth muscle cells of pulmonary vessels, histiocytes, and mucous glandular cells of the bronchial wall stained faintly with anti-VEGF antibody. In contrast, sarcoid granulomas stained positive with VEGF immunostaining (Fig. 1A, C). The reaction product was localized in activated alveolar macrophages of alveolar spaces (Fig. 1E), multinuclear giant cells and epitheloid cells of sarcoid granulomas (Fig. 1A, C), which were all labelled positive simultaneously by anti-CD 68 antibodies (data not shown). VEGF staining was localized intracytoplasmatically in these cells. Lymphocytes showed no detectable immunoreaction with anti-VEGF antibody.

There was a heterogenous staining pattern of the anti-VEGF antibody in the three open lung biopsies. A variety of staining intensities among granulomas was observed in the same biopsy. Subjectively the most intensive staining was found in early, still epitheloid-cell-rich granulomas without fibrosis. Here the VEGF-staining pattern was more homogeneous than in mature epitheloid granulomas with multinuclear giant cells (Fig. 1A). In mature granulomas the reaction product was visualized predominantly in multinuclear giant cells and epitheloid cells (Fig. 1C). Some granulomas were not stained by the anti-VEGF antibody and showed fibrotic features on elastica–van Gieson staining. An increased expression of VEGF was detected in pleural granulomas in open lung biopsy specimens. Normal mesothelial cells showed no detectable VEGF immunostaining near the granuloma. Endothelial cells of microvessels near the granulomas stained positive with the anti-VEGF antibody.

In total, 23 of the 33 specimens showed a positive immunoreaction against VEGF in granulomas and in alveolar macrophages of the associated alveolitis. In 10 cases granulomas exhibited no VEGF staining, and 8 of these simultaneously showed negative CD-68 immunostaining.

Increased expression of *flt* receptor was detected by immunohistochemistry in activated alveolar macrophages and in macrophage-derived cellular components of granulomas, such as epitheloid cells and multinuclear giant cells (Fig. 1G, H). Normal mesothelial cells, bronchial epithelium, and lymphocytes did not react positively with the anti-*flt* antibody, but a weak reaction was observed in vascular endothelial cells. In total, 7 of the 33 biopsies stained positive for *flt.*

Twenty biopsy specimens were analysed for VEGF transcript with nonradioactive in situ hybridization. Stromal cells, vascular endothelial cells and bronchial epithelial cells did not label positive for VEGF mRNA. In contrast, cells containing high levels of VEGF transcripts (Fig. 1B, D) were identified as epitheloid cells by CD 68 immunostaining. Multinuclear giant cells also showed increased levels of VEGF mRNA (Fig. 10D). Many of the activated alveolar macrophages near to the epitheloid granulomas labelled strongly for VEGF mRNA (Fig. 1F) and immunohistochemically showed a simultaneously increased production of the cytokine (Fig. 1E). Epitheloid-cell-rich granulomas located in visceral pleura contained mRNA of VEGF in epitheloid cells, but none of the neighbouring mesothelial cells labelled positive for VEGF mRNA. No detectable signals were found either in granulomas or in activated alveolar macrophages in control cases labelled with pBR322 DNA.

Discussion

Sarcoidosis is a systemic granulomatous disorder, nearly always with pulmonary manifestations. Active pulmonary sarcoidosis is characterized by inflammatory alveolitis and epitheloid granuloma formation, which may progress to fibrosis in 20% of the patients [18]. The sarcoid granuloma is composed of macrophages, monocytes, epitheloid cells, multinucleated giant cells, and activated T-lymphocytes. Epitheloid cells are derived and matured from circulating monocytes with a higher capacity for extensive protein synthesis than nonactivated monocytes or macrophages [16]. Activated macrophages from sarcoid patients release a large amount of interleukin-1 and are thus able to induce enhanced replication and activation of T-lymphocytes [10]. Otherwise, tumour-associated activated macrophages are known to be a major source of various cytokines that regulate angiogenic processes, such as basic fibroblast growth factor or VEGF [12]. Although severe angiitis is associated infrequently with sarcoidosis, endothelial cell proliferation and neoformation of a diffuse capillary network have often been found within and adjacent to sarcoid granulomas. Meyer et al. reported an enhanced angiogenesis-inducing capacity of macrophages separated from bronchoalveolar lavage (BAL) of patients with pulmonary sarcoidosis. The effect was significantly greater in BAL cells of patients with active than in patients with inactive sarcoidosis [14].

We found increased transcription and production of VEGF in activated alveolar macrophages in inflammatory alveolitis associated with pulmonary sarcoidosis. Moreover, activated-matured forms of macrophages, such as epitheloid cells and multinuclear giant cells, showed a high signal intensity of VEGF mRNA on nonradioactive in situ hybridization. Overexpression of the growth factor could be illustrated in these cells by immunohistochemistry. In contrast, fibrotic, cell-poor granulo-

mas were negative according to VEGF immunostaining. Activated alveolar macrophages and monocyte-derived cellular elements of sarcoid granulomas, such as epitheloid cells and multinuclear giant cells, expressed the *flt* receptor at high levels simultaneously. In this context it has to be mentioned that we analysed only specimens from patients with active disease (clinical symptoms and/or radiological signs).

For the first time, our results demonstrated a coexpression of VEGF and *flt* in pulmonary sarcoidosis. These findings are in accordance with previous reports describing an increased expression of VEGF in activated macrophages of various origins including malignant tumours and cases of rheumatoic arthritis [17]. VEGF has been found to be chemotactic for macrophages in malignant tissues and is involved in the recruitment of monocytes from the blood-stream into necrosis tumour areas [22]. Similarly, VEGF may have a dual effect in sarcoidosis, acting as a chemoattractant and activating agent for monocytes and also inducing proliferating of endothelial cells and angiogenesis within and adjacent to epitheloid granulomas. Its chemoattractive effect may initiate a large amount of circulating monocytes to aggregate in alveolar and in interstitial spaces of the lung. Moreover, a vascular hyperpermeability-inducing effect of VEGF may sustain this process, enhancing the migration of cellular elements through the capillary wall. The observed heterogeneity of the VEGF-staining pattern may be explained by a temporary down-regulation of the growth factor, by an uncharacterized mechanism to finish the process. Whether or not the angiogenic effect of VEGF is only a “side effect” of granuloma formation or is of pathophysiological importance in the fibrotic process remains unclear. The simultaneously increased *flt* receptor expression of activated macrophages and monocytes suggests that VEGF may act through the *flt* receptor pathway in granuloma formation.

Several steroids are known to decrease VEGF production directly [9]. The findings presented here may support the hypothesis that the relevant effect of steroid therapy in suppressing acute symptoms of sarcoidosis may be due to the down-regulation of VEGF expression of activated monocytes and macrophages.

Acknowledgements The authors thank Professor W. Risau for providing the human VEGF cDNA and Mrs. Sabine Böhm, Ms. Susanne Schaub and Ms. Anja Mauermann for their excellent technical assistance. This work was supported by the German Academic Exchange Service (DAAD).

References

1. Adams DO (1983) The biology of the granuloma. In: Ioachim HL (ed) Pathology of granulomas. Raven Press, New York, pp 1–20
2. Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Marmè D (1996) Migratin of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor *flt*-1. Blood 87:3336–3343
3. Berse B, Brown LF, van de Water L, Dvorak HF, Senger DR (1992) Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages and tumors. Mol Biol Cell 3:211–220
4. Clauss M, Gerlach H, Brett J, Wang F, Familletti PC, Pan YCE, Olander JV, Connolly DT, Stern D (1990) Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. J Exp Med 172:1535
5. Clauss M, Weich H, Breier G, Knies U, Rockl W, Walterberger J, Risau W (1996) The vascular endothelial growth factor receptor *flt*-1 mediates biological activities. Implications for a functional role of placenta growth factor in monocyte activation and chemotaxis. J Biol Chem 271:17629–17634
6. Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, Siegel NR, Leingruber RM, Feder J (1989) Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 84:1470–1478
7. Cordell JL, Falini B, Erber WN (1984) Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal antialkaline phosphatase (APAAP) complexes. J Histochem Cytochem 32:219–229
8. Gruber BL, Marchese MJ, Kew R (1995) Angiogenic factors stimulate mast-cell migration. Blood 86:2488–2493
9. Heiss JD, Papavassiliou E, Merrill MJ, Nieman L, Knightly JJ, Walbridge S, Edwards NA, Oldfield EH (1996) Mechanism of dexamethasone suppression of brain tumor-associated vascular permeability in rats. J Clin Invest 98:1400–1408
10. Hunninghake GW (1984) Release of interleukin-1 by alveolar macrophages of patients with active pulmonary sarcoidosis. Am Rev Respir Dis 129:569–572
11. Johnson GD, De Noguera C, Aranjo JGM (1986) A simple method for reducing the fading of immunfluorescence during microscopy. J Immunol Methods 43:349–350
12. Leek RD, Harris AL, Lewis CE (1994) Cytokine networks in solid tumors: regulation of angiogenesis. J Leukoc Biol 56:423–435
13. McLaren J, Prentice A, Charnock-Jones DS, Millican SA, Müller KH, Sharkey AM, Smith SK (1996) Vascular endothelial growth factor is produced by peritoneal fluid macrophages in endometriosis and is regulated by ovarian steroids. J Clin Invest 98:482–489
14. Meyer KC, Kaminski MJ, Calhoun WJ, Auerbach R (1989) Enhanced capacity of bronchoalveolar lavage cells from patients with pulmonary sarcoidosis to induce angiogenesis in vivo. Am Rev Respir Dis 140:1446–1449
15. Mikami R, Sekiguchi M, Ryuzin Y (1986) Changes in peripheral vasculature of various organs of patients with sarcoidosis – possible role of microangiography. Heart Vessels 2:129–139
16. Müller K-M (1996) Sarkoidose – Pathologische-anatomische Befunde. In: Sarkoidose – Fachbeiträge zum Krankheitsbild auf dem Weg der Synthetik. Deutsche Sarkoidose Vereinigung, Meerbusch, pp 72–98
17. Nagashima M, Yoshino S, Ishiwata T, Asano G (1995) Role of vascular endothelial growth factor in angiogenesis of rheumatoid arthritis. J Rheumatol 22:1624–1630
18. O’Connor C, Odlum C, van Breda A, Power C, Fitzgerald M (1988) Collagenase and fibronectin in bronchoalveolar lavage fluid in patients with sarcoidosis. Thorax 43:393–400
19. Plate KH, Breier G, Weich HA, Risau W (1992) Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vitro. Nature 359:845–848
20. Rosen Y (1993) Sarcoidosis. In: Dail DH, Hammar SP (eds) Pulmonary pathology. Springer, Berlin Heidelberg New York, pp 615–637
21. Sheid B (1992) Angiogenic effects of macrophages isolated from ascitic fluid aspirated from women with advanced ovarian cancer. Cancer Lett 62:153–158
22. Shen H, Clauss M, Ryan J, Schmidt AM, Tijberg P, Borden L, Connolly D, Stern D, Kao J (1993) Characterization of vascular permeability factor/vascular endothelial growth factor receptors on mononuclear phagocytes. Blood 81:2767–2773
23. Wiethege T, Voss B, Pohle T, Fisseler-Eckhoff A, Müller KM (1991) Localization of elastase and tumor necrosis factor alpha mRNA by non-radiactive in situ hybridization in cultures of alveolar macrophages. Pathol Res Pract 187:912–915