Virchows Arch (1995) 427:385–393

ORIGINAL ARTICLE

D. Gindre · S. Peyrol · M. Raccurt · P. Sommer
R. Loire · J.A. Grimaud · J.F. Cordier

Fibrosing vasculitis in Wegener's granulomatosis: ultrastructural and immunohistochemical analysis of the vascular lesions

Received: 23 February 1995 / Accepted: 23 August 1995

Abstract This study of two cases of pulmonary Wegener's granulomatosis (WG) focuses on the ultrastructural aspects of the vascular wall injury and on the immunohistochemical characterization of the perivascular connective matrix. The iterative waves of endothelial cell necrosis and regeneration are demonstrated by the multilamellar appearance of the basal lamina. Neutrophils infiltrate the vessel wall and myofibroblasts are recruited to injured vessels. The perivascular connective matrix associates basement-membrane like and fibrillar material with fibrin deposits. The initiation of the fibrosing process was assessed by the visualization of matrix molecules involved in targeting (p-fibronectin), organizing (cellular fibronectin and tenascin) and stabilizing (lysyl-oxidase) the fibrogenic activity. These elementary lesions affect different levels of the vascular tree, and capillaritis is involved in the extension of the pathological process. Lysyl-oxidase labelling reveals the fibrosing front which is located on the border of dense fibrosis. The markers of fibrosing activity disappear in the areas of fibrosis following vasculitis and/or ischaemic necrosis and/or granulomatosis. Vasculitis plays a major role in both the genesis and progression of the fibrosis observed in the late stage of WG.

Key words Wegener's granulomatosis · Vasculitis · Fibrosis

Work supported by a grant of the Association pour la Recherche sur le Cancer (ARC 2057)

D. Gindre · R. Loire
Département de Pathologie, Hôpital Cardiovasculaire et Pneumologique, Lyon, France

S. Peyrol (✉) · M. Raccurt · P. Sommer · J.A. Grimaud
Unité de Pathologie des Fibroses – CNRS URA 1459, Institut Pasteur de Lyon, Avenue Tony Garnier, F-69365 Lyon Cedex 07, France

J.F. Cordier
Service de Pneumologie, Hôpital Cardiovasculaire et Pneumologique, Lyon, France

Introduction

Wegener's granulomatosis (WG) is a necrotizing granulomatous vasculitis of unknown origin affecting mainly the upper and lower respiratory tracts and the kidney where it causes focal and segmental glomerulonephritis ([56]; and for clinical studies [13, 18, 59]). The frequency of "limited" WG [9] without renal involvement, and the rare occurrence of Wegener's granulomatosis restricted to the kidney, has suggested that the respiratory tract might be the initial target of the unknown causal agent [15]. Although necrosis remains a prominent feature of pulmonary WG [19, 40] the lesions may evolve to fibrosis located around the necrotizing granulomatous areas. The vasculitis affects different levels of the vascular tree including small arteries and capillaries [54]. The leucocytoclastic capillaritis defined as fibrinoid necrosis of capillary walls may represent an early lesion of the disease. Histologically, it is difficult to individualize these early microvascular lesions in the necrotizing foci surrounded by fibrosis. An ultrastructural approach allows a better understanding of the evolution of the microvascular injury [16].

Endothelial necrosis, basement membrane denudation and fibrin deposition constitute the initial vascular lesion. The presence of intravascular lysed leucocytes, in early lesions, suggested a role for circulating mediators such as cytophilic antibodies [7, 16, 41]. Circulating antineutrophil cytoplasmic antibodies (ANCA) can be identified during the active phase of the disease [21, 22] and are helpful markers in monitoring the course of the disease, and the response to treatment [4, 5, 51]. The target autoantigen was recently decoded as proteinase 3, a neutral serine protease of human neutrophil azurophil granules [8, 14, 29, 42].

Our observations were focused on the pulmonary microvasculature. Open lung biopsies were investigated at the ultrastructural level in order to characterize the cell alterations in the vessel wall and the infiltrating inflammatory and fibrogenic cells. Immunohistochemistry allowed us to define the particular matrix component profile of perivascular fibrosis. In addition, the initiation of

the fibrosing process was assessed by the visualization of matrix molecules involved in targeting (plasma-fibronectin), organizing (cellular-fibronectin, tenascin) and stabilizing (lysyl-oxidase) the fibrogenic activity.

Materials and methods

Two cases of pulmonary WG without renal involvement were diagnosed on the basis of the classical histological criteria observed in open lung biopsies.

Clinical data

Case 1

The patient was a 56-year-old man who developed a cavity of the right pulmonary upper lobe associated with sinusitis. One year later, the pulmonary cavity had enlarged and he developed vasculitic purpura, arthralgia and lacrymal duct inflammation. A right upper lobectomy was done 11 months after the onset of symptoms and the finding of the pulmonary cavity. The patient received classical treatment with cyclophosphamide and corticosteroids and complete remission was obtained. However, he later relapsed and diffuse cytoplasmic ANCA were found.

Case 2

This patient was a 45-year-old man who presented with fever, weight loss and dyspnoea. Chest X-ray showed bilateral condensation of the pulmonary upper lobes. Open lung biopsy was done 1 month after the onset of symptoms. This patient died of fulminant pulmonary vasculitis within a few days after lung biopsy in 1985 and no ANCA test was available.

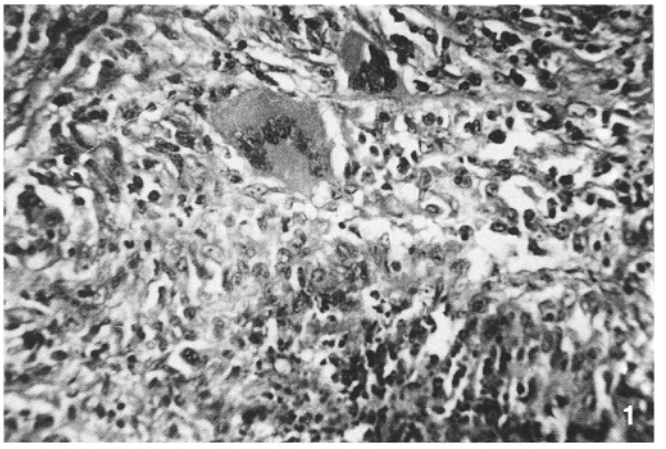
The lung biopsies were processed for immunohistochemistry and electron microscopy.

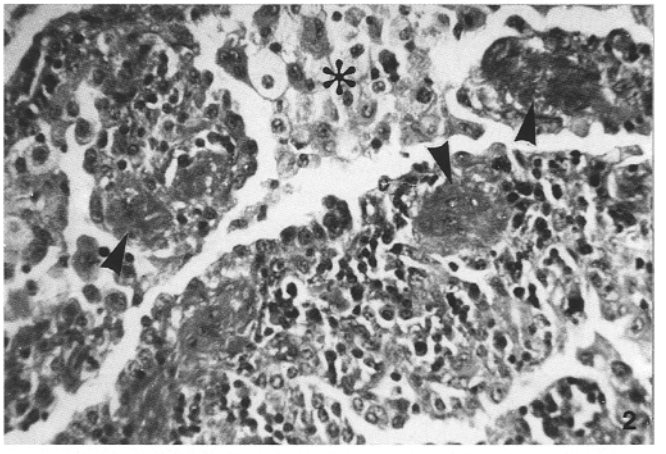
Electron microscopy

Part of the biopsy was immediately covered with 2% glutaraldehyde-0.1 M sodium(Na)cacodylate/hydrochloric acid(HCl) pH 7.4, cut into 1 mm^3 pieces and fixed for 2 h at 4° C in the same fixative. After washing three times (3 h) at 4° C in 0.2 M Nacacodylate/HCl pH 7.4, the specimen was post-fixed in 1% osmium tetroxide – 0.15 M Nacacodylate/HCl pH 7.4 for 1 h at 4° C, dehydrated in graded ethanols and embedded in epon. Areas showing microvessel alterations were selected on semi-thin sections stained with methylene blue-azure II. Consecutive ultra-thin sections were contrasted with methanolic uranyl acetate and lead citrate, and observed with a Philips EM 300 microscope.

Immunohistochemistry

An indirect immunofluorescence method using matrix component markers and fluorescein isothiocyanate-conjugates was applied to 5 µm cryostat sections of fresh frozen tissue. An indirect immunoperoxidase method using streptavidin-biotin complex (Immu-Mark, ICN Biomedicals) was applied to 3 µm paraffin-embedded tissue sections. Antibodies against human type I, type III, type IV collagens, bovine type III procollagen and murine laminin (polyclonal), were from the Institut Pasteur, Lyon, France; monoclonal anti-human cellular fibronectin – cell attachment site antibodies (clone II 3E3 A002) and polyclonal anti-human tenascin antibodies (A 107) were from Telios Pharmaceuticals, California; polyclonal anti-lysyl oxidase antibodies were a gift from P. Sommer [50].





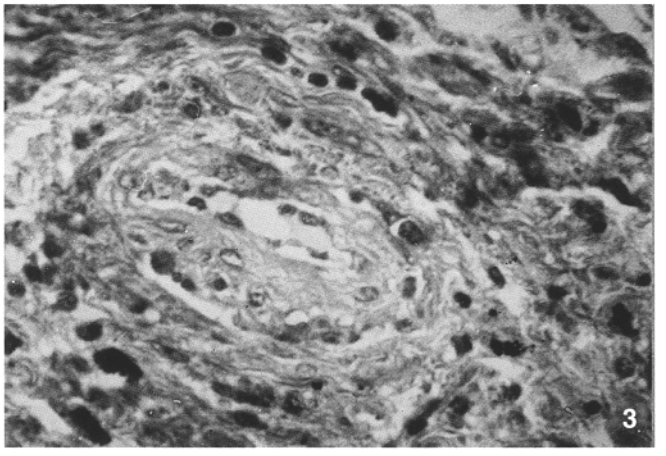
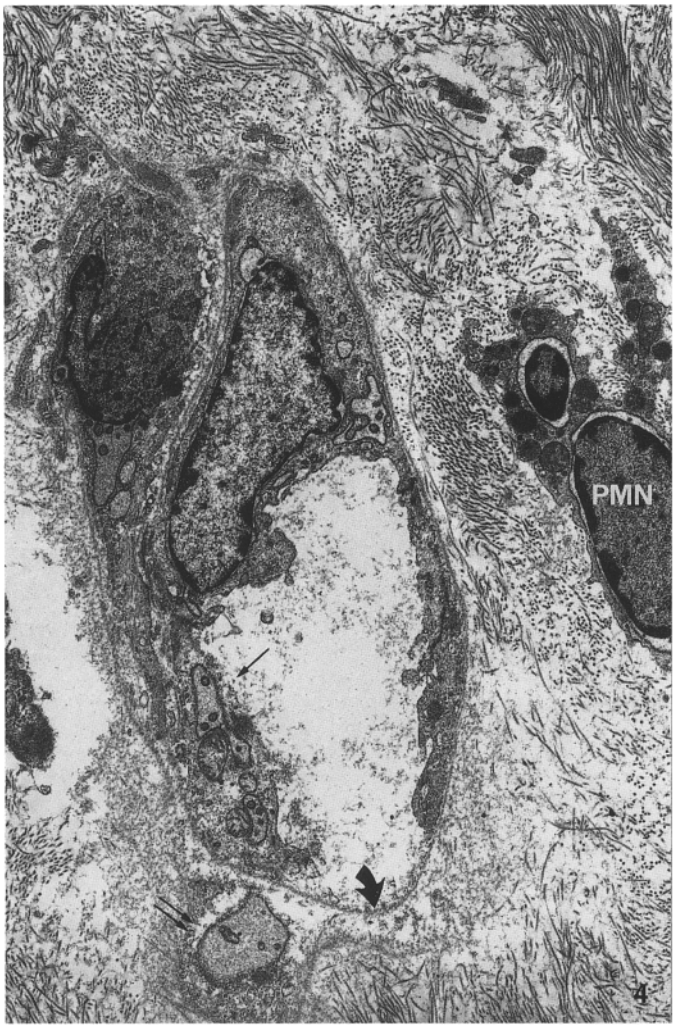
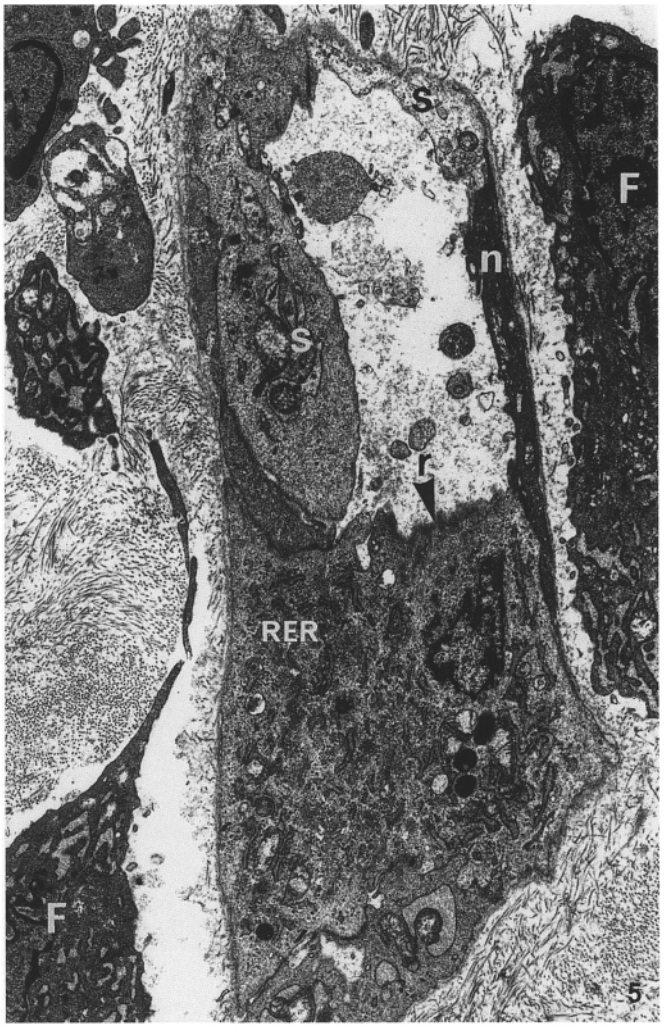


Fig. 1 Giant cell in granulomatous tissue with palisading disposition of histiocytes at the periphery of a necrotic focus. Haematoxylin-phloxin-saffron (HPS), original magnification (OM) ×250

Fig. 2 Thickening and inflammation of the alveolar septa in the perilesional pulmonary parenchyma: thrombosis of capillaries (*arrow*) and alveolitis (*asterisk*). HPS, OM ×250

Fig. 3 Small artery: dramatic reduction of the lumen associated with fibrotic thickening of the vessel wall. HPS, OM ×250





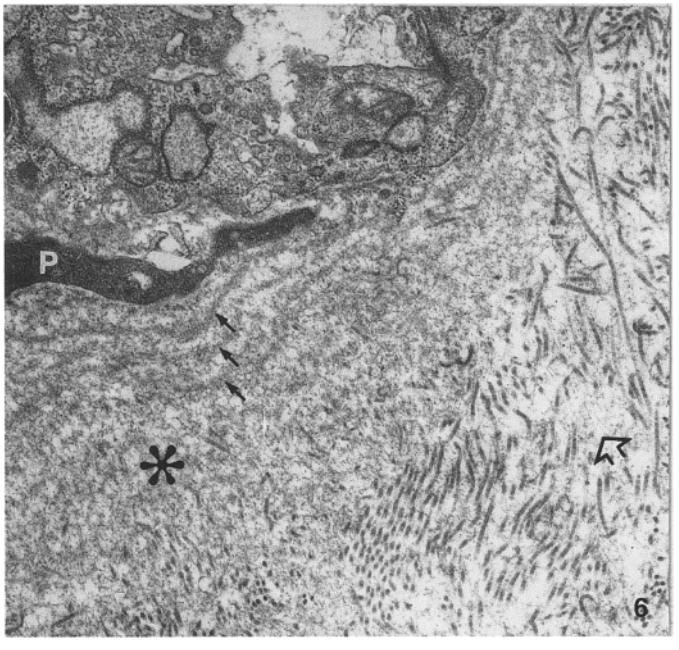
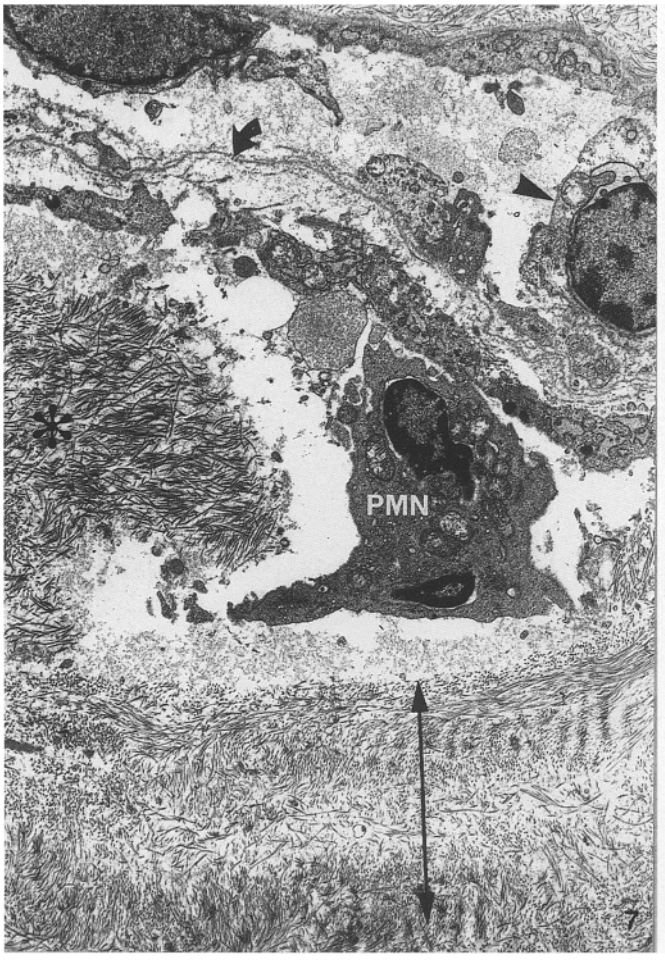


Fig. 4 Ultrastructural aspect of an altered capillary among fibrosis: partial denudation of the subendothelial basal lamina (*thick arrow*) related to endothelial cell (*thin arrow*) and pericyte (*double thin arrow*) lysis; presence of a neutrophil polymorphonuclear cell (*PMN*) in pericapillary fibrosis. ×6500

Fig. 5 Heterogeneous aspect of the capillary endothelium: necrosis (*n*); clarifying swelling (*s*); regeneration (*r*); presence of activated fibroblasts (*F*) in pericapillary fibrosis. *RER* Rough endoplasmic reticulum. ×5400

Fig. 6 Multilaminated subendothelial basal lamina (*small arrows*) with accumulation of microfibrillar material (*asterisk*). Perivascular fibrosis presents a loose organization and is composed of thin collagen fibres and microfibrils. *P* densified pericyte. ×10000



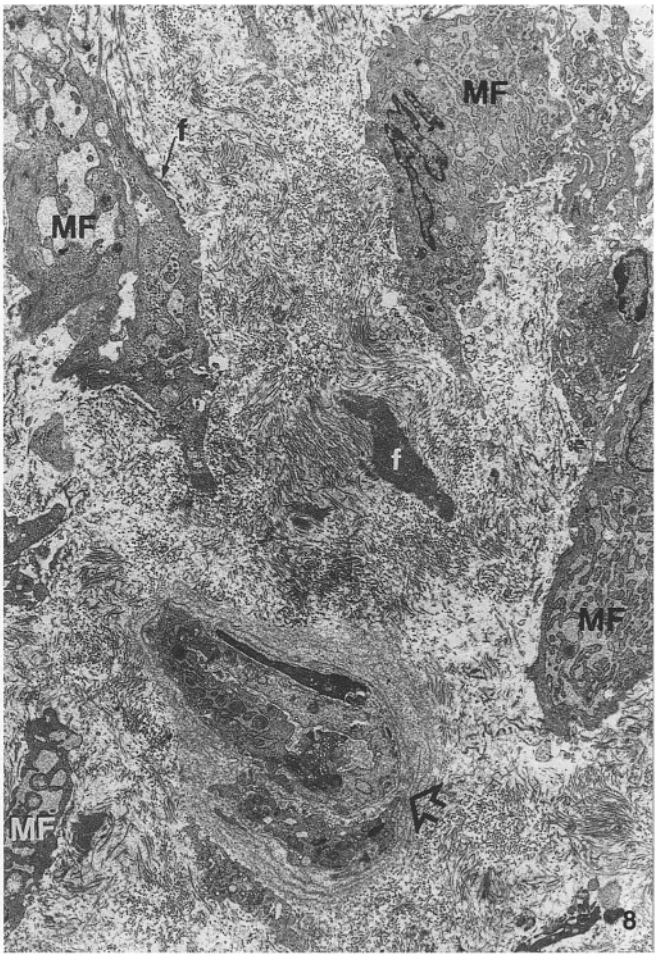


Fig. 7 Irregular fibrillar collagen deposits (*asterisk*) in the perivascular connective wall of a capillary showing severe endothelial cell alterations: denudation of the subendothelial basal lamina (*arrow*); swelling of an endothelial cell showing a pyknotic nucleus (*arrowhead*). Alternation of collagen layers with different orientations (*double-ended arrow*). ×5400

Fig. 8 Fibrotic environment of an altered microvessel (*open arrow*): recruitment of myofibroblasts (*MF*) in fibrosis in process of organization with rudimentary collagen fibre bundles enclosing fibrin spots (*f*); a fibrin lamina is shown in close apposition to a myofibroblast. ×4400

Results

Histopathology

Case 1

Large necrotic fields were surrounded by inflammatory granulomas with palisading epithelioid cells and scattered giant cells (Fig. 1). Arteriolitis appeared as mural inflammatory infiltration or as paucicellular fibrotic wall thickening leading to lumen occlusion. In the parenchymal areas contiguous with perinecrotic granulomas where the lung architecture was preserved, non specific macrophagic alveolitis, septal inflammatory thickening and capillary thrombosis were observed (Fig. 2). Fibrosis was closely intermingled with granulomas and vasculitis.

Case 2

There were small necrotic areas embedded in dense fibrosis and the granulomatous inflammatory process was moderate with few giant cells. Vasculitis displayed the evolution stages from inflammation to fibrosis, but was mainly fibrotic (Fig. 3). At the border of the fibrotic lesions, capillaritis and alveolitis were constantly associated.

Electron microscopy

The endothelial lamina was interrupted in places because of endothelial cell lysis leading to denudation of the basal lamina (Fig. 4). At the same site swelling, necrosis and regeneration of endothelial cells were seen (Fig. 5). The regenerating endothelial cells had dense thick perinuclear cytoplasm with highly developed endoplasmic reticulum [ER; rough ER (RER) and Golgi complex],

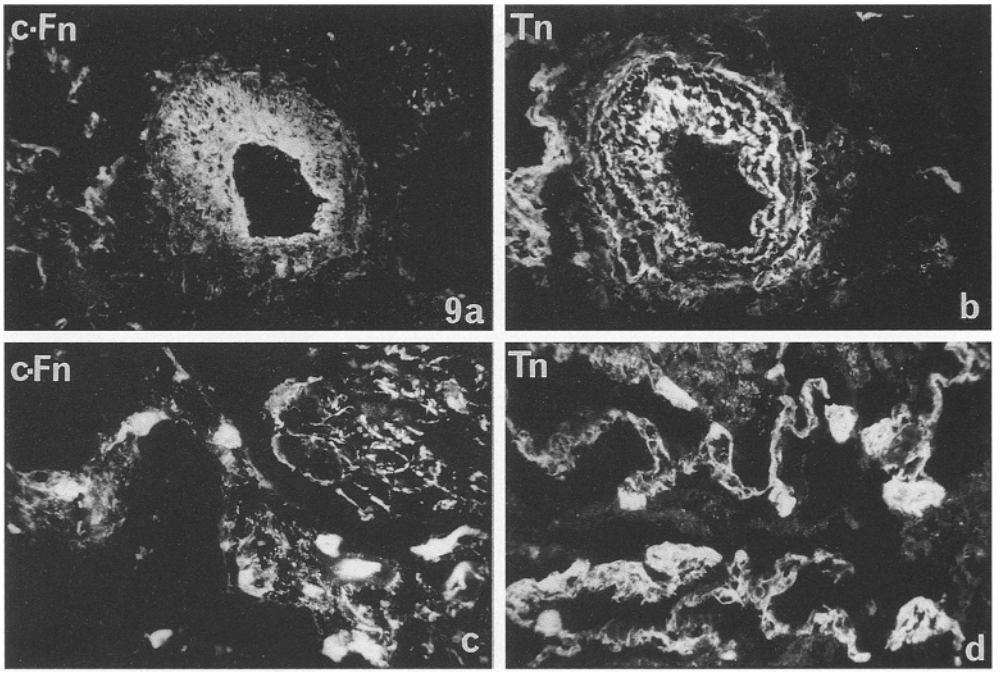


Fig. 9a–d Immunofluorescence staining of cellular-fibronectin (*c-FN*) and tenascin (*Tn*) in a small artery (**a**, **b**) and in septal capillaries (**c**, **d**). Serial sections emphasize the codistribution of c-FN (**a**) and Tn (**b**) in the thickened arteriolar intima. In lung parenchyma c-FN (**c**) and Tn (**d**) intensely decorate alveolar capillary walls and adjacent thickened alveolar septa, **a**, **b**, **d** ×250; **c**. ×400

diffusely disposed organelles and cytoskeleton; the periluminal cytoplasmic processes were short with large plates of intercellular junctions. Pericytes showed degenerative and regenerative changes (Fig. 4–6). Adjacent to the endothelial and pericytic lesions, the basal lamina had a fuzzy aspect and was often stratified; in that case, a mixed microfibrillar basement membrane-like layer with a variable thickness had accumulated in contact with it (Fig. 6). The vessel wall was infiltrated by inflammatory cells, mainly polymorphonuclear cells, often associated with anarchic foci of collagen fibres (Fig. 7).

In the perivascular connective space, an abundant polymorphic fibrotic matrix displayed an increasing density with distance from the vessel wall: adjacent to the basal laminae, thin periodic collagen fibres and microfibrils formed a loose fibrillar matrix, and appeared to be anchored perpendicularly to the fuzzy basement membrane material (Fig. 6). Farther off, collagen fibres acquired a parallel arrangement and showed different modes of organization: in the vicinity of the altered vessel wall, they sometimes formed successive layers with different orientations (Fig. 7); beyond, they formed short curved bundles without strict reciprocal disposition which were assembled in large collagen fields with dense organization (Fig. 8). One peculiarity of these perivascular fibrotic deposits was the regular small diameter and the short length of the constitutive collagen fibres. Fibrin deposition was conspicuous in this fibrotic matrix; it appeared either as electron dense laminae often in close contact with fibroblasts or as large clusters embedded in perivascular fibrosis distant from the vascular lesion (Fig. 8). Numerous activated fibroblasts were seen in the perivascular area. They were characterized by a highly developed RER and, for some of them, by a myoid cytoskeleton indicating a myofibroblastic phenotype (Fig. 8).

Immunohistochemistry

In small arteries, an overexpression of cellular-fibronectin and tenascin, restricted to the vessel wall, was concentrated in the subendothelial area and was diffuse in the medial inflammatory infiltrate (Fig. 9a, b). Both labellings faded in dense fibrotic areas. In the alveolar septa, at the border of the fibrotic lesion, bright deposits of tenascin and cellular-fibronectin emphasized alveolar capillaritis (Fig. 9c, d).

The perivascular basement membranes (visualized by their main components type IV collagen and laminin), appeared irregularly thickened, and showed a multilamellar aspect focally (Fig. 10a). In the late fibrotic stage, vestigial basement membrane deposits suggested a vascular origin for the fibrotic process (Fig. 10b). Large bright fields of procollagen type III were disposed in the thickened vessel walls and extended into the adjacent septal interstitium (Fig. 10c). These deposits were superimposed on type I collagen (Fig. 10e) and all together constituted the fibrillar framework of perivascular fibrosis. In established stenotic vascular lesions, type I collagen was seen in concentric layers (Fig. 10f). In the late stage, in the thickened septa where vascular lesions had evolved, type I and type III collagens were equally and diffusely codistributed with an associated fading of the procollagen type III labelling, the initial vascular structure was only suggested by the whirpool disposition of the extensive fibrotic deposits (Fig. 10d). Lysyl-oxidase expression was variable: when present, lysyl-oxidase cir-

cumscribed the inflammatory mural infiltrate and formed a fibrosing front between inflammation and dense peripheral fibrosis (Fig. 11a). Lysyl-oxidase labelling vanished in fibrotic vessels (Fig. 11b).

Discussion

The histological lesions of both cases correspond to the mixed type of the classification proposed by Yoshikawa [62], which associates acute lesions (alveolitis and vasculitis), cicatricial nodular areas and persistant granulomatous lesions. In WG, vasculitis is characterized by alterations concerning all the constitutive structural elements of the vessel wall: endothelium, basal lamina, pericytes and associated perivascular connective matrix. These structural elements are successively involved during the progression of the pathological process from early endothelial injury to late perivascular fibrosis.

Endothelial necrosis is the first vasculitic event. It has been suggested that the intravascular sequestration and/or lysis of activated polymorphonuclear leucocytes is causative [14, 16]. The major involvement of the alveolar capillaries is due to the fact that the lung capillary bed is the principal site of neutrophil sequestration, allowing inflammatory mediators to promote further neutrophil-endothelial interactions [35]. At the site of endothelial necrosis, endothelial continuity is restored by regeneration attested by the phenotype of endothelial cells with dense cytoplasm, suggestive and structural remodelling [24, 49]. This regenerative phenotype succeeds migration and proliferation of adjacent undamaged endothelial cells in large injuries of the endothelium [23, 45] suggesting that in WG, necrosis affects large plates of endothelial cells. Furthermore, the coexistence at the same site of degenerative and regenerative cells may indicate the persistence of the causal factors. In WG the lysosomal releasates from activated neutrophils may be involved in the extension of tissue destruction to the subendothelial connective matrix, with their wide spectrum of matrix protein degrading enzymes affecting basement membrane collagen (type IV), interstitial collagens (Type I-III-V) and elastin [41, 57, 61].

The vessel wall components are dramatically remodelled in the disease. The basal lamina appears denuded but without discontinuity. Its multilamellar aspect gives evidence of successive waves of basal lamina neogenesis which might be related to the iterative endothelial regeneration [28, 55]. Endothelial cells are known to be responsible for fibronectin and basement membrane component synthesis [25]. At the site of injury the basement mem-

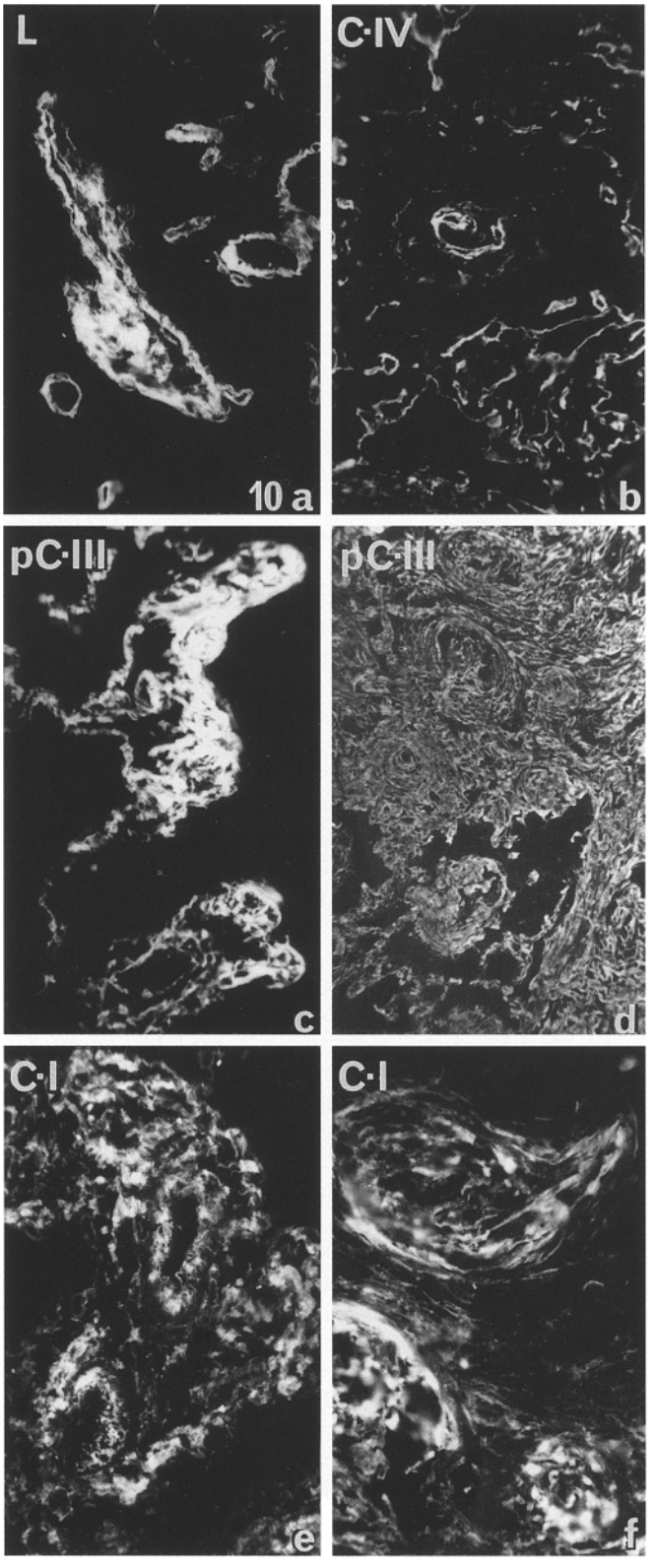


Fig. 10a–f Immunofluorescence staining of matrix components in early (**a**, **c**, **e**) and end stage (**b**, **d**, **f**) perivascular fibrotic lesions. **a** Laminin staining emphasizes the multi-layered perivascular basal laminae (×250). **b** Type IV collagen staining visualizes vestigial basal lamina in the centre of a fibrotic vascular lesion (×250). **c** procollagen type III staining clearly discriminates thickened alveolar septa at the sites of capillary lesions (×250). **d** fading of procollagen type III staining is observed in late fibrotic lesions. It reveals perivascular fibrosis which contributes to severe reduction of the alveolar space (×100). **e** In early vascular lesions, type I collagen is deposited in the thickened vascular wall (×250). **f** In late fibrotic lesions, type I collagen shows a multilamellar concentric disposition (×250))

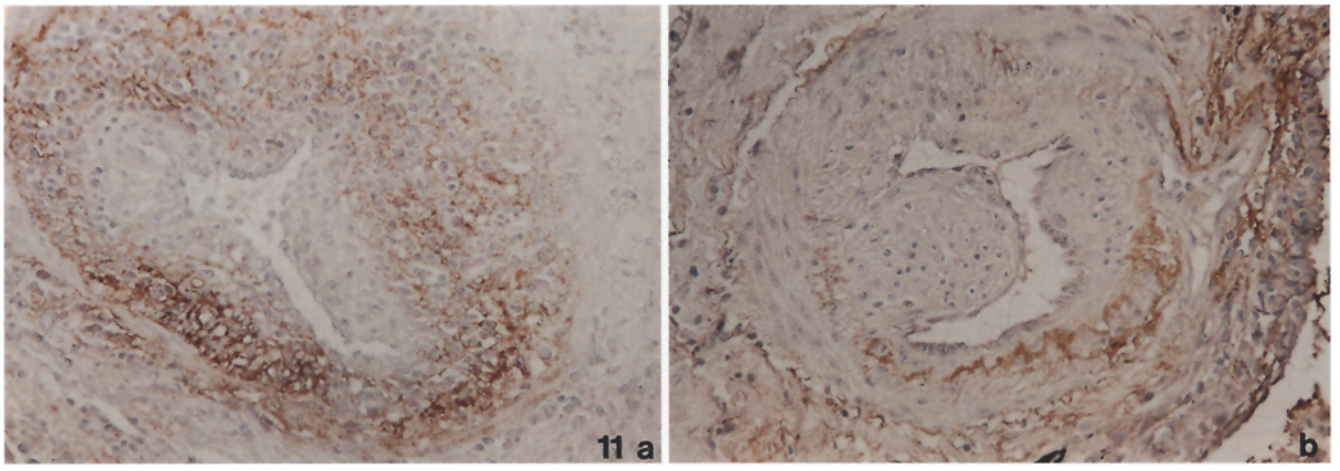


Fig. 11a, b Immunoperoxidase staining of lysyl-oxidase (LO). **a** In early perivascular lesions, LO is expressed in the peripheral crown superimposed to the inflammatory infiltrate (×250). **b** In the late fibrotic stage, LO staining is restricted to remodelled elastic fibres of the perivascular elastic lamina (×250)

brane has a fuzzy aspect suggesting a local overproduction of additional microfibrillar matrix components including fibronectin and type III collagen, as proved by immunostaining. The pericytes also undergo degenerative and regenerative changes. In this situation, they might be able to synthesize, as endothelial cells, a larger amount and spectrum of matrix proteins than in normal conditions, contributing to basement membrane and vessel wall thickening [53]. Reciprocally cellular-fibronectin may promote endothelial migration and regeneration [26]. These elementary lesions affect different levels of the vascular trees including small muscular arteries, small veins and septal capillaries. They are attested by the presence of cellular-fibronectin and tenascin in the subendothelial area. The cellular-fibronectin isoform, here characterized by its cell attachement site, discriminates early cell-matrix interactions since it is supposed to play a role in the nucleation of fibronectin fibrillogenesis which precedes further collagen deposition and organization [26, 37]. Tenascin, an extracellular matrix glycoprotein strongly implicated in epithelio-mesenchymal interactions during fetal development [2, 3, 32], has a more restricted distribution mainly in subepithelial and subendothelial areas in adults [36]. Its stromal induction in some pathological conditions such as wound healing [11, 38, 58] and tumour stroma reaction [10, 33] attests to an organizing activity in newly forming extracellular matrices. In WG, its perivascular overexpression could indicate an active matrix remodelling of the vascular wall, initiated by the endothelial lesion. The capillary alterations, located at the border of the normal parenchyma with achieved fibrotic areas, may represent local progression of the pathological process. They are responsible for the alveolar haemorrhage usually present in WG [6, 4, 12].

P-fibronectin isoform associated with the altered endothelial lining appears to be the key factor in the recruitment of neutrophils and histiocytes into the perivascular area. The inflammatory cell infiltrate in the vessel wall evidently plays a major role in the local production of cytokines responsible for oedema, coagulation pathway activation with fibrin deposition [27] and fibroblastic cell recruitment [31, 34, 44, 46]. Fibrin deposits related to the endothelial lesion may evoke a defect in the fibrin resorbing process by phagocytes. Their embedding in the matrix suggests an extensive fibrosing process in this area. Codistributed fibrin and fibronectin in perivascular areas are known to promote the recruitment, migration and differentiation of interstitial cells [20, 60]. Fibroblasts are observed closely adherent to fibrin. The high cellularity of the perivascular connective matrix probably results from the proliferation of the interstitial cell population. The ultrastructural characters in the fibroblastic cells are consistent with their activation status including active protein synthesis and the development of a myoid cytoskeleton supports their migrating ability [1, 47]. Most fibroblastic cells are myofibroblasts, thus suggesting an intense recruitment towards the lesion.

A correlation may be established between the codistribution of some matrix components (procollagen type III, type I and type III collagens and fibronectin) and their ultrastructural mode of organization: collagen fibres appear short, thin, curved and loosely assembled into a polymorphic fibrillar matrix. This aspect is consistent with an early stage of the fibrotic process [43]. Furthermore lysyl-oxidase, the enzyme of the earliest step of type I collagen and elastin cross-linking [30] which has been recently described as an early marker of collagen fibril stabilization [50], displays focal expression at the periphery of vascular inflammation. Lysyl-oxidase detection localizes the front of fibrosing activity at this site and confirms that vasculitis generates fibrosis. The disappearance of lysyl-oxidase expression, procollagen type III, fibronectin and basement membrane components and the concomitant concentric dense organization of type I and type III collagens evoke the progressive maturation of the perivascular fibrotic deposits [52]. Lysyl-oxidase thus allows us to distinguish the active fibrosing areas from late stage fibrosis.

Among the pulmonary lesions which characterize WG, fibrosing vasculitis may be considered as a fundamental lesion initiated by endothelial aggression. This point represents the main difference with regard to idiopathic fibroproliferative diseases (IPF and liver cirrhosis) which are characterized by the permanent disregulation of the fibrocompetent cell activity, leading to extensive fibrosis. In contrast successive waves of endothelial necrosis coincide with the evolutive bursts of WG and generate reactive fibrosis with limited spatio-temporal extension proven by the transient perivascular expression of lysyl-oxidase. The link between endothelial necrosis and circulating ANCA has been established in vitro [17, 48] and in vivo [39]. Vasculitis-induced perivascular fibrosis takes part in the fibrosing course of WG and further generates an ischaemic process which is obviously an additional factor in the generation of necrosis and fibrosis.

Acknowledgements The authors are very grateful to Liliane Loncke for daily reliable assistance in patient sample collection and electron microscope preparation. Sylviane Guerret and Daniel Hartmann for the well controlled quality of the matrix protein antibodies, Gerard Joly for the electron microscope management and competent photography technique and Agnès Belhabri for the careful and elegant secretarial work

References

1. Adler KB, Low RB, Leslie KO, Mitchell J, Evans JN (1989) Contractile cells in normal and fibrotic lung. Lab Invest 60:473–482
2. Aufderheide E, Ekblom P (1988) Tenascin during gut development: appearance in the mesenchyme, shift in molecular forms, and dependence on epithelial-mesenchymal interactions. J Cell Biol 107:2341–2349
3. Aufderheide E, Chiquet-Ehrismann R, Ekblom P (1987) Epithelial mesenchymal interactions in the developing kidney lead to expression of tenascin in the mesenchyme. J Cell Biol 105:599–608
4. Baltaro RJ, Hoffman GS, Sechler JMG,Suffredini AF, Shelhamer JH, Fauci AS, Fleisher TA (1991) Immunoglobulin G antineutrophil cytoplasmic antibodies are produced in the respiratory tract of patients with Wegener's granulomatosis. Am Rev Respir Dis 143:275–278
5. Beer DJ (1992) ANCAs a weigh. Am Rev Respir Dis 146:1128–1130
6. Bosch X, Font J, Mirapeix E, Revert L, Ingelmo M, Urbano-Marquez A (1992) Antimyeloperoxidase autoantibody-associated necrotizing alveolar capillaritis. Am Rev Respir Dis 146:1326–1329
7. Brasile L, Kremer JM, Clarke JL, Cerilli J (1989) Identification of an autoantibody to vascular endothelial cell-specific antigens in patients with systemic vasculitis. Am J Med 87:74–80
8. Braun MG, Csernok E, Gross WL, Muller-Hermelink HK (1991) Proteinase 3, the target antigen of anticytoplasmic antibodies circulating in Wegener's granulomatosis: immunolocalisation in normal and pathologic tissues. Am J Pathol 139:831–838
9. Carrington CB, Liebow AA (1966) Limited form of angeitis and granulomatosis of Wegener's type. Am J Med 41:497–527
10. Chiquet-Ehrismann R, Mackie EJ, Pearson CA, Sakakura T (1986) Tenascin: an extracellular matrix protein involved in tissue interactions during fetal development and oncogenesis. Cell 47:131–139
11. Chuong CM, Chen HM (1991) Exhausted expression of neural cell adhesion molecules and tenascin (cytotactin) during would healing. Am J Pathol 138:427–440
12. Colby TV (1989) Diffuse pulmonary haemorrhage in Wegener's granulomatosis. Semin Respir Med 10:136–140
13. Cordier JF, Valeyre D, Guillevin L, Loire R, Brechot JM (1990) Pulmonary Wegener's granulomatosis. A clinical and imaging study of 77 cases. Chest 97:906–912
14. Csernok H, Lüdemann J, Gross WL, Bainton D (1990) Ultrastructural localization of proteinase 3, the target antigen of anti-cytoplasmic antibodies circulating in Wegener's granulomatosis. Am J Pathol 137:1113–1120
15. DeRemee RA, McDonald TJ, Harrison EG Jr, Coles DT (1976) Wegener's granulomatosis anatomic correlates: a proposed classification. Mayo Clin Proc 51:777–781
16. Donald KJ, Edwards RL, McEvoy JDS (1976) An ultrastructural study of the pathogenesis of tissue injury in limited Wegener's granulomatosis. Pathology 8:161–169
17. Falk RJ, Terell RS, Charles LA, Jennette JC (1990) Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. Proc Natl Acad Sci USA 87:4115–4119
18. Fauci AS, Wolff SM (1973) Wegener's granulomatosis: studies in eighteen patients and a review of literature. Medicine (Baltimore) 52:535–561
19. Fienberg R (1989) Morphological and immunohistologic study of the evolution of the necrotizing palisading granuloma of pathergic (Wegener's) granulomatosis. Semin Respir Med 10:126–132
20. Grinnel F, Feld M, Minter D (1980) Fibroblast adhesion to fibrinogen and fibrin: Requirement for cold insoluble globulin (plasma fibronectin). Cell 19:517–525
21. Gross WL (1989) Wegener's granulomatosis. Sarcoidosis 6:15–29
22. Harrison DJ, Simpson R, Khaebanda R, Abernethy VE, Nimmo G (1989) Antibodies to neutrophil cytoplasmic antigens in Wegener's granulomatosis and other conditions. Thorax 44:373–377
23. Haudenschild CC, Schwartz SM (1979) Endothelial regeneration. II. Restitution of endothelial continuity. Lab Invest 41:405–418
24. Huttner I, Walker C, Gabbiani G (1985) Aortic endothelial cell during regeneration. Remodeling of cell junctions, stress fibers, and stress-fiber membrane attachment domains. Lab Invest 53:287–302
25. Hynes RO (1990) Expression of fibronectins by cells in culture. In: Rich A (ed) Fibronectins. Springer, New York Berlin Heidelberg, pp 65–67
26. Hynes RO (1990) Fibronectin biosynthesis and fibrillogenesis. The role of the cells. In: Rich A (ed) Fibronectins. Springer, New York Berlin Heidelberg, pp 192–194
27. Idell S, James KK, Gillies C, Fair DS, Thrall RS (1989) Abnormalities of pathways of fibrin turnover in lung lavage of rats with oleic acid and bleomycin-induced lung injury support alveolar fibrin deposition. Am J Pathol 135:387–399
28. Imayama S, Ikawa T, Urabe H (1986) Basal lamina of the dermal capillary; concentric multilamination following regeneration processes. J Dermatol 13:456–459
29. Jenne DE (1990) Wegener's autoantigen decoded. Nature 346:520
30. Kagan HM, Trackman PC (1991) Properties and function of lysyl-oxidase. Am J Respir Cell Mol Biol 5:206–210
31. Kelley J (1990) Cytokines of the lung. Am Rev Respir Dis 14:765–788
32. Koch M, Wehrle-Haller B, Baumgartner S, Spring J, Brubacher D, Chiquet M (1991) Epithelial synthesis of tenascin at tips of growing bronchi and graded accumulation in basement membrane and mesenchyme. Exp cell Res 194:297–300
33. Koukoulis GK, Gould VE, Bhattacharyya A, Gould JE, Howeedy AA, Virtanen I (1991) Tenascin in normal, reactive, hyperplastic and neoplastic tissues: biologic and pathologic implications. Hum Pathol 22:636–643

34. Kovacs E (1991) Fibrogenic cytokines: The role of immune mediators in the development of scar tissue. Immunol Today 12:17–23
35. Lien DC, Henson PM, Capen RL, Henson JE, Hanson WL, Wagner WW, Worthen GS (1991) Neutrophil kinetics in the pulmonary microcirculation during acute inflammation. Lab Invest 65:145–159
36. Lightner VA, Gumkowski F, Bigner DD, Erickson HP (1989) Tenascin/Hexabrachion in human skin: biochemical identification and localization by light and electron microscopy. J Cell Biol 108:2483–2493
37. Limper AH, Roman J (1992) Fibronectin: a versatile matrix protein with roles in thoracic development, repair and infection. Chest 101:1663–1673
38. Mackie EJ, Halfer W, Liverani D (1988) Induction of tenascin in healing wounds. J Cell Biol 107:2757–2767
39. Malik AB, Horgan MJ (1987) Mechanisms of thrombin-induced lung vascular injury and edema. Am Rev Respir Dis 136:467–470
40. Mark EJ, Matsubara O, Tan-Liu NS, Fienberg R (1988) The pulmonary biopsy in the early diagnosis of Wegener's (pathergic) granulomatosis. A study based on 35 open lung biopsies. Hum Pathol 19:1065–1071
41. Movat HZ, Wasi S (1985) Severe microvascular injury by lysosomal releasates of human polymorphonuclear leukocytes. Am J Pathol 121:404–417
42. Nölle B, Specks U, Lüdemann J, Rohrbach MS, DeRemee RA, Gross WL (1989) Anticytoplasmic autoantibodies: their immunodiagnostic value in Wegener granulomatosis. Ann Intern Med 111:28–40
43. Peyrol S, Takiya C, Cordier JF, Grimaud JA (1986) Organization of the connective matrix of the sarcoid granuloma: evolution and cell-matrix interactions. Tenth international conference on sarcoidosis and other granulomatous disorder. Ann NY Acad Sci 465:268–285
44. Peyrol S, Cordier JF, Grimaud JA (1990) Intra-alveolar fibrosis of idiopathic bronchiolitis obliterans-organizing pneumonia: cell-matrix patterns. Am J Pathol 137:155–170
45. Reidy MA, Schwartz SM (1981) Endothelial regeneration. III. Time course of intimal changes after small defined injury to rat aortic endothelium. Lab Invest 44:301–308
46. Rennard SI, Hunninghake GW, Bitterman PB, Crystal RG (1985) Production of fibronectin by the human alveolar macrophage: mechanism for recruitment of fibroblasts to sites of tissue injury in interstitial lung diseases. Proc Natl Acad Sci USA 78:7147–7151
47. Sappino AP, Schürch W, Gabbiani G (1990) Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulations. Lab Invest 63:144–161
48. Savage COS, Pottinger BE, Gaskin G, Pusey CD, Pearson JD (1992) Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. Am J Pathol 141:335–342
49. Schwarz MI, Mortenson RL, Colby TV, Waldron JA, Lynch JA, Hutt MP, Cherniack M, King TE (1993) Pulmonary capillaritis: the association with progressive irreversible airflow limitation and hyperinflation. Am Rev Respir Dis 148:507–511
50. Sommer P, Gleyzal C, Raccurt M, Delbourg M, Serrar M, Joazeiro P, Peyrol S, Kagan H, Trackman PC, Grimaud JA (1993) Transient expression of lysyl-oxidase by liver myofibroblasts in murine schistosomiasis. Lab Invest 69:460–470
51. Specks U, DeRemee R (1989) Significance of antibodies to cytoplasmic components of neutrophils. Thorax 44:369–379
52. Takiya C, Peyrol S, Cordier JF, Grimaud JA (1983) Connective matrix organization in human pulmonary fibrosis. Virchows Arch [B] 44:223–240
53. Tan EML, Glassberg E, Olsen DR, Noveral JP, Unger GA, Peltonen J, Chu ML, Levine E, Sollberg S (1991) Extracellular matrix gene expression by human endothelial and smooth muscle cells. Matrix 11:380–387
54. Travis WD, Hoffman GS, Leavitt RY, Pass HI, Fauci A (1991) Surgical pathology of the lung in Wegener's granulomatosis. Am J Surg Pathol 15:315–333
55. Vracko R, Benditt EP (1970) Capillary basal lamina thickening: Its relationship to endothelial cell death and replacement. J Cell Biol 47:281–291
56. Wegener F (1936) Über generalisierte, septische Gefäßerkrankungen.Verh Dtsch Pathol Ges 29:202–210
57. Weiss SJ (1989) Tissue destruction by neutrophils. N Engl J Med 320:365–376
58. Whitby DJ, Longaker MT, Harrison MR, Adzick NS, Ferguson MW (1991) Rapid epithelialisation of fetal wounds is associated with the early deposition of tenascin. J Cell Sci 99:583–586
59. Wolff SM, Fauci AS, Horn RG, Dale DC (1974) Wegener's granulomatosis. Ann Intern Med 81:513–525
60. Woods A, Couchman JR (1988) Focal adhesions and cell-matrix interactions. Col Rel Res 8:155–182
61. Worthen GS, Haslett C, Rees AJ, Gumbay RS, Henson JE, Henson PM (1987) Neutrophil-mediated pulmonary vascular injury. Am Rev Respir Dis 136:19–28
62. Yoshikawa Y, Watanabe T (1986) Pulmonary lesions in Wegener's granulomatosis: a clínico-pathologic study of 22 autopsy cases. Hum Pathol 17:401–410