Brief Definitive Report

Vascular Permeability Factor/Endothelial Growth Factor (VPF/VEGF): Accumulation and Expression in Human Synovial Fluids and Rheumatoid Synovial Tissue

By Roy A. Fava, Nancy J. Olsen,* George Spencer-Green, Kiang-Teck Yeo,‡ Tet-Kin Yeo,‡ Brygida Berse,‡ Robert W. Jackman,‡ Donald R. Senger,‡ Harold F. Dvorak,‡ and Lawrence F. Brown‡

*From the Research Service (151), Veterans Affairs Medical Center, White River Junction, Vermont 05009; the Department of Medicine, Dartmouth Medical School, Hanover, New Hampshire 03756; the *Division of Rheumatology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232; and the ‡Department of Pathology, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215*

Summary

Vascular permeability factor (VPF, also known as vascular endothelial growth factor or VEGF), is a potent microvascular permeability enhancing cytokine and a selective mitogen for endothelial cells. It has been implicated in tumor angiogenesis and ascites fluid accumulation. Since development of the destructive synovial pannus in rheumatoid arthritis (RA) is associated with changes in vascular permeability (synovial fluid accumulation), synovial cell hyperplasia, and angiogenesis, we examined synovial fluids (SFs) and joint tissue for the expression and local accumulation of VPF/VEGF. VPF/VEGF was detected in all of 21 synovial fluids examined and when measured by an immunofluorimetric assay, ranged from 6.9 to 180.5 pM. These levels are biologically significant, since <1 pM VPF/VEGF can elicit responses from its target cells, endothelial cells. Levels of VPF/VEGF were highest in rheumatoid arthritis fluids (n = 10), with a mean value (± SEM) of 59.1 ± 18.0 pM, vs. 21.4 ± 2.3 pM for 11 SFs from patients with other forms of arthritis (p = 0.042). In situ hybridization studies that were performed on joint tissues from patients with active RA revealed that synovial lining macrophages strongly expressed VPF/VEGF mRNA, and that microvascular endothelial cells of nearby blood vessels strongly expressed mRNA for the VPF/VEGF receptors, flt-1 and KDR. Immunohistochemistry performed on inflamed rheumatoid synovial tissue revealed that the VPF/VEGF peptide was localized to macrophages within inflamed synovium, as well as to microvascular endothelium, its putative target in the tissue. Together, these findings indicate that VPF/VEGF may have an important role in the pathogenesis of RA.

Vascular permeability factor (VPF) was originally identified as a 34–42-kD peptide secreted by tumor cells (1–3). VPF renders the microvasculature hyperpermeable to circulating macromolecules with a potency some 50,000 times greater than histamine; it is found in animal and human tumor effusions where it may contribute to fluid accumulation (4). This protein is also a selective endothelial cell mitogen and promotes angiogenesis in vivo, hence its additional name, vascular endothelial growth factor (VEGF) (5). To emphasize the importance of both these activities, the term VPF/VEGF is used here.

In addition to its association with tumors, VPF/VEGF is expressed in a limited number of sites in normal tissues (6, 7) and is upregulated in keratinocytes during wound healing, a process that involves microvascular hyperpermeability and angiogenesis (8). Expression patterns of a VPF/VEGF-receptor, flt-1, correspond very well to sites of VPF/VEGF expression in normal tissues during murine embryonic development and during cutaneous wound healing (9).

By inducing increased microvascular permeability and endothelial cell growth, VPF/VEGF may facilitate angiogenesis and tumor stroma formation (6, 10). In fact, neutralization of VPF/VEGF with antibodies not only suppresses extravasation of plasma proteins in vivo (2) but also inhibits tumor growth in vivo (11). In situ hybridization studies have demonstrated VPF/VEGF expression in a variety of trans-

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/94/07/0341/06 $2.00
Volume 180 July 1994 341–346

plantable animal and autochthonous human tumors. Moreover, the expression of specific endothelial cell receptors for VPF/VEGF (KDR and flt-1) is simultaneously upregulated in nearby blood-vessel endothelium in tumors (1).

The hyperplastic synovial pannus in rheumatoid arthritis resembles a solid tumor in certain ways, especially in its intrinsic cellular proliferation, its invasive properties and the association of angiogenesis with the development of this highly vascularized tissue (12). Pharmacological inhibition of angiogenesis is known to block experimental arthritis (collagen-induced arthritis) (13). Inflammatory arthritis is also characterized by the accumulation of synovial fluids (SFs), an effusion rich in plasma proteins. These facts taken together suggested to us that VPF/VEGF might have a role in the pathogenesis of arthritis.

Materials and Methods

Human SFs and Joint Biopsies. SFs were collected by aspiration from patients with a variety of arthritides. Total white cell counts were performed and the fluids were centrifuged at 2,500 rpm for 15 min to pellet cells and particulate matter. Aliquots of the supernatants were stored at −70°C. Differential cell counts were not performed. Synovial tissue was from wrist synovectomies (n = 4) or hips (n = 2) for the rheumatoid arthritis specimens and from a hip (n = 1) for osteoarthritis. Patients ranged from 40–65 yr of age.

Assays for VPF/VEGF and Rheumatoid Factor in SFs. VPF/VEGF was measured with a sensitive and highly specific immunofluorimetric assay, similar to a sandwich ELISA (14). Briefly, antibody directed against a COOH-terminal peptide of human VPF/VEGF was immobilized on microtitre plates; SFs containing VPF/VEGF (or standards or control buffer) were allowed to bind, and the plates washed. Bound VPF/VEGF was detected with a second antibody directed against an NH_2-terminal peptide of VPF/VEGF, labeled with Eu^{3+}-chelate. Fluorescence was detected as described (14). Human recombinant VPF/VEGF, kindly provided by Dr. Steven R. Ledbetter (The Upjohn Company, Kalamazoo, MI), was employed as a standard.

Rheumatoid factor was assayed by ELISA as previously described using as a standard, a monoclonal IgM (κ) isolated from a cryoprecipitate (15, 16). Rheumatoid factor assays were used to confirm diagnoses and to assess possible interference with measurement of VPF/VEGF.

In Situ Hybridization (ISH) and Immunohistochemistry (IH). Reagents were prepared with diethypyrocarbonate-treated PBS. Synovial biopsies were fixed in 4% paraformaldehyde-phosphate buffered saline, pH 7.4 (PBS), for 2–4 h at 4°C and were then transferred to 30% sucrose-PBS overnight at 4°C, frozen in OCT compound (Miles Diagnostics, Elkhart, IN) and stored at −70°C. ISH was performed on 6 μm frozen tissue sections. Selection and preparation of the antisense and sense (control) single stranded riboprobes for VPF/VEGF, and for the VPF/VEGF endothelial cell receptors flt-1 and KDR, have been described (17). The antisense VPF/VEGF probe hybridizes specifically with a region of VPF/VEGF mRNA common to all four known VPF/VEGF splicing variants (18, 19).

The tissue specimens processed for ISH were found to also be suitable for immunohistological studies. An affinity-purified rabbit antibody to the NH_2-terminal peptide (amino acid residues 1–26) of human VPF/VEGF was used in IH (and as the second antibody in the VPF/VEGF immunoassay) and its preparation has been described (10, 20). This antipeptide antibody specifically binds VPF/VEGF in ELISA assays and on immunoblots, blocks VPF/VEGF activity, and, when linked to agarose, selectively absorbs VPF/VEGF from solution (20); because of its specificity, it has become the antibody of choice for demonstrating VPF/VEGF in tissue sections by IH (10, 17). KP-1, an antibody specific for human macrophages, was purchased from Dako Corp. (Carpinteria, CA). An antipeptide antibody specific for fibrin was the kind gift of Dr. Gary Matsueda (Massachusetts General Hospital, Boston, MA); this antibody is directed against the NH_2-terminal portion of the fibrin β chain and does not recognize fibrinogen (21). IH was performed with an avidin-biotin peroxidase conjugate protocol (10, 22). Normal rabbit IgG diluted to an equivalent protein concentration was used as a control instead of the primary antibody.

Western Blotting of Human VPF/VEGF and Synovial Tissue Extract. Human recombinant VPF/VEGF was purchased from R&D Systems, Inc. (Minneapolis, MN). An extract of human rheumatoid arthritis (RA) synovial tissue was made by homogenizing 30–40 mg of tissue in 3 ml ice cold 20 mM Tris, 150 mN NaCl, 20 mM EDTA buffer, pH 7.5. The extracts were centrifuged 5 min at 12,000 rpm in a microfuge to remove particulates, the protein content determined, and aliquots were subjected to 12% SDS-PAGE under reducing conditions.

Into individual lanes were loaded either 8 μg total synovial tissue extract, 12 ng human recombinant VPF/VEGF (with BSA carrier protein), or 8 μg of tissue extract spiked with 12 ng VPF/VEGF. The proteins were subjected to SDS-PAGE and transferred to nitrocellulose paper. Replicate lanes were examined by silver staining. After the transfer the nitrocellulose "blot" was blocked with 10% milk protein, incubated with a 1:200 dilution of the affinity purified anti-VPF/VEGF in PBS, and then subjected to biotin/avidin enhanced peroxidase detection (Vector Laboratories Inc., Burlingame, CA). The substrate was 3,3-diaminobenzidine.

Results and Discussion

Immunoassay of VPF/VEGF in Synovial Fluids. Immunofluorimetric analysis revealed detectable VPF/VEGF in all SFs examined from 21 patients with various forms of arthritis; values ranged from 6.9 to 180.5 pM (Table 1). The highest levels of VPF/VEGF were found in SFs taken from patients with clinically well-documented RA. Although there was

Table 1. *VPF/VEGF Concentration in Synovial Fluids*

Diagnosis	Range	Mean ± SEM	Median
	pM	*pM*	
RA* (n = 10)	(6.9–180.5)	59.1 ± 19.0	42.2
OA (n = 3)	(24.9–36.0)	29.5 ± 3.4	27.5
Gout (n = 3)	(8.5–24.8)	17.4 ± 4.8	18.9
Misc.‡ (n = 5)	(12.9–23.5)	17.7 ± 3.3	22.1

All of seven fluids that had VPF/VEGF levels >40 pM were from patients with RA (p <0.001; Fisher's exact test). OA, osteoarthritis.
* The mean of the VPF/VEGF values in RA fluids was significantly different from the means of SFs with all other diagnoses (p = 0.042).
‡ Miscellaneous diagnoses included: psoriatic arthritis (n = 1), avascular necrosis (n = 1), trauma (n = 1), and unknown (n = 2).

overlap, mean levels of VPF/VEGF were significantly higher in the SFs of patients diagnosed as having RA than in those of patients with other forms of arthritis ($p = 0.04$). However, the levels of VPF/VEGF measured in all of the joint fluids studied may well have biological significance since VPF/VEGF levels as low as ~0.2 pM are effective in activating cultured endothelial cells (23). Total white cell counts were performed on all SFs but these did not correlate well with VPF/VEGF levels ($r = 0.174$; $p = 0.5$). Differential cell counts were not performed; it is not yet known whether VPF/VEGF levels correlate well with a specific cell type, as previously shown for monocyte/macrophages in inflammatory fluids (4).

Histology of Synovial Biopsies: Expression of VPF/VEGF and Its Receptors. Synovial biopsies were available from six patients with RA and from one with osteoarthritis. Biopsies from three of the patients with RA revealed hyperplastic, inflamed synovial tissue (pannus), characterized histologically by extensive infiltration of lymphocytes, macrophages and plasma cells, fibrin deposition, and increased numbers of new blood vessels lined by hypertrophic endothelium (Fig. 1). Fibrin, positively identified by immunohistochemistry, was most abundant superficially near the synovial surface and to a lesser extent in deeper zones (Fig. 1, *E* and *F*). Synovial tissue biopsied from the three other patients with a clinical diagnosis of RA revealed no significant inflammation; i.e., inactive disease. Joint tissue from the single case of osteoarthritis was hyperplastic but contained only rare inflammatory cells.

In situ hybridization performed on two of the three patients with active RA revealed a population of synovial lining cells that labeled intensely for VPF/VEGF mRNA; positive cells had morphological features of synovial macrophages (Fig. 2, *A* and *B*). Occasional inflammatory cells more deeply situated in pannus, also having morphological features of macrophages, labeled for VPF/VEGF but less intensely than macrophages closer to the synovial surface. In addition, vascular endothelial cells of small pannus blood vessels labeled intensely for the mRNAs of both VPF/VEGF receptors, flt-1 and KDR (Fig. 2, *E–H*). In synovium from a third RA patient, the lining layer was not present; but, an inflammatory infiltrate

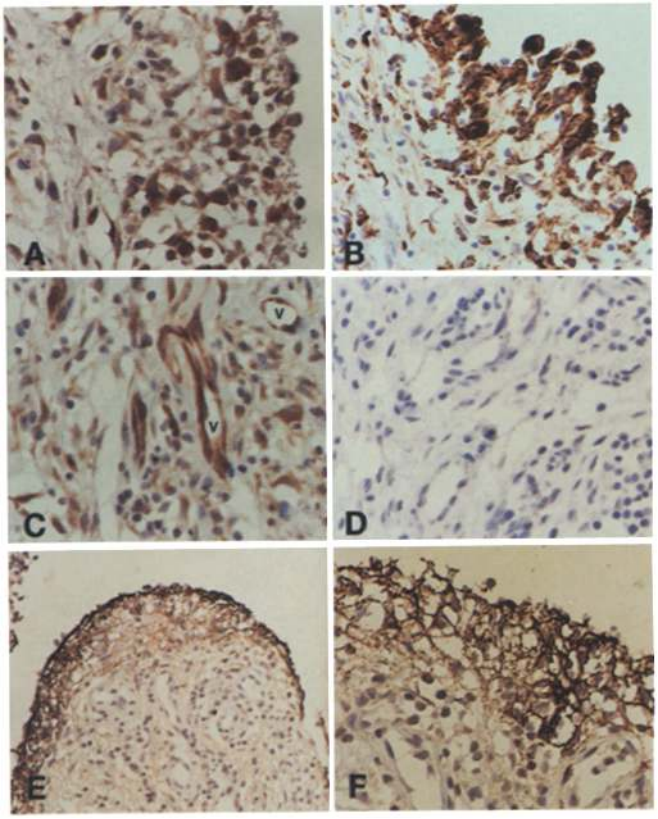

Figure 1. Immunohistochemical staining of inflamed synovial tissue (pannus) from a patient with active RA. A population of cells near the synovial surface stains strongly for VPF/VEGF (*A*) and also for the macrophage marker KP-1 (*B*). Endothelial cells lining adjacent small vessels (two are labeled v) stain with antibodies against VPF/VEGF (*C*) but do not stain with a control antibody (*D*). Low power (*E*) and higher power (*F*) photomicrographs demonstrate fibrin deposits concentrated at the synovial surface. Original magnifications: *A–D* and *F*, ×279; *E*, ×107.

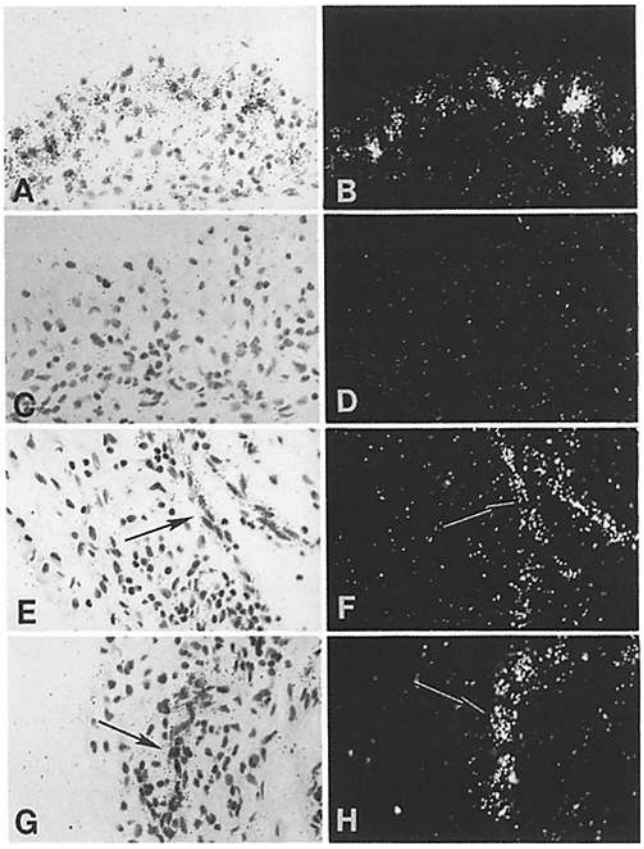

Figure 2. In situ hybridization of inflamed synovial tissue from a patient with active RA, illustrated in photomicrographs of the same fields in brightfield (*A*, *C*, and *G*) and darkfield (*B*, *D*, and *H*). (*A* and *B*) Intense labeling with a probe for VPF/VEGF mRNA of a population of synovial lining cells having the morphology of macrophages. (*C* and *D*) Background with control VPF/VEGH sense probes was consistently low. (*E–H*) Endothelial cells in small vessels near the synovial surface label strongly for mRNAs of the VPF/VEGF receptors flt-1 (*E* and *F*) and KDR (*G* and *H*). Original magnifications: ×288.

was found deep in the tissue in which the vascular endothelium was found to express KDR mRNA.

In contrast, in the noninflamed synovia available from three other patients with RA and in the single case of osteoarthritis, specific labeling was not observed with antisense probes to VPF/VEGF or to VPF/VEGF receptor mRNAs. Background labeling with sense (control) probes was consistently low in all tissues examined (Fig. 2, *C* and *D*).

Immunohistochemical Detection of VPF/VEGF Protein in Pannus. In biopsies from patients with active RA, immunostaining with antibodies to VPF/VEGF revealed a population of cells similar in morphology, frequency and distribution to those identified as expressing VPF/VEGF mRNA; this cell population also stained for KP-1, a marker for cells of macrophage lineage (Fig. 1, *A* and *B*) (24).

Endothelial cells of small blood vessels in pannus also stained for VPF/VEGF in the patients with active RA (Fig. 1, *C* and *D*). As noted above, endothelium of vessels in the same specimens did not express VPF/VEGF mRNA (as detected by in situ hybridization) but did express mRNA for both of the VPF/VEGF-receptors examined.

In sections of synovial tissue from a patient with osteoarthritis, there was no significant inflammatory cell infiltration; nonetheless, very rare synovial macrophages stained for VPF/VEGF (not shown). Background immunostaining with IgG control antibodies was always insignificant.

To demonstrate the specificity of the anti-VPF/VEGF used in the immuno-staining procedure shown in Fig. 1, a Western blot was performed with pure recombinant VPF/VEGF and synovial tissue extracts (Fig. 3). VPF/VEGF was not present in sufficient amount to detect in the tissue extract, as expected, since overall only a relatively small proportion of cells of the tissue contained VPF/VEGF mRNA (as shown in Fig. 1). However, 12 ng of purified VPF/VEGF was readily detected by the anti-VPF/VEGF antibody, either alone (Fig. 3, lane *5*) or combined with the synovial tissue extract (Fig. 3, lane *6*). Even though present at at 600-fold excess over VPF/VEGF, there was no significant recognition of other tissue proteins by the anti-VPF/VEGF in this procedure.

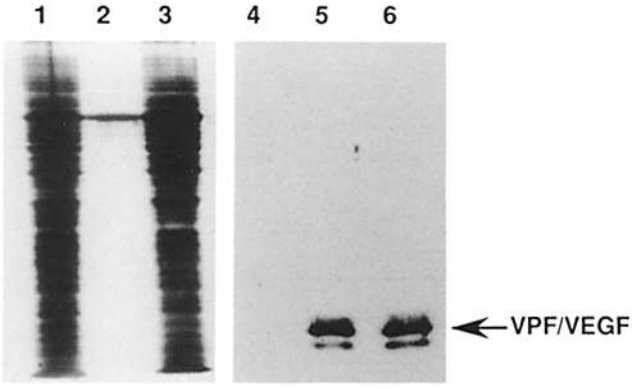

Figure 3. Western blotting of synovial tissue extract and human recombinant VPF/VEGF. Lanes *1–3*, silver stained 12% SDS-PAGE; Lanes *4–6*, nitrocellulose transfer of replicate gel immunostained with affinity purified anti-VPF/VEGF. Lanes (*1* and *4*), 8 μg synovial tissue extract; lanes (*2* and *5*), 12 ng VPF/VEGF with BSA carrier protein; lanes (*3* and *6*), 8 μg synovial tissue extract plus 12 ng VPF/VEGF.

VPF/VEGF Expression and the Pathogenesis of RA. The data presented indicate that VPF/VEGF mRNA is expressed by synovial macrophages in patients with active RA and furthermore, that VPF/VEGF protein is found in the cytoplasm of these macrophages, as well as in association with endothelial cells lining nearby blood vessels. In addition, mRNAs for the two best characterized VPF/VEGF receptors, flt-1 and KDR, are expressed by microvascular endothelial cells that stain with antibodies to VPF/VEGF. These findings were absent in patients with clinically inactive disease.

The simplest explanation of these data is that in active RA synovial macrophages are stimulated to synthesize and secrete VPF/VEGF; secreted VPF/VEGF binds to local blood vessel endothelium by way of specific receptors and also extravasates into the joint cavity. Binding of VPF/VEGF renders the endothelium of small blood vessels hyperpermeable to plasma proteins, which leak into the joint cavity, producing a protein-rich effusion. Extravasated fibrinogen has been previously demonstrated in synovial tissues where it may contribute to the fibrin deposition found in rheumatoid synovium (but not osteoarthritis) (25, 26). This fibrin may serve as a potent stimulus for angiogenesis in this region of the RA synovium, as has been already shown in other tissues (27). A similar sequence of events is encountered in wound healing, where tissue fibrin deposits are replaced by highly vascular granulation tissue that closely resembles pannus (8). These events also echo those found in many tumors, where expression of both VPF/VEGF and its receptors (flt-1 and KDR) are strikingly upregulated and have been implicated in tumor angiogenesis and stroma formation (1, 28).

VPF/VEGF was also found in the SFs of patients with other forms of arthritis besides RA. Although the amounts of VPF/VEGF present were significantly lower than those found in RA, they were still more than sufficient to effect biological activity. It is noteworthy that the fibrin deposition demonstrated in rheumatoid synovium is not found in other forms of arthritis (26), and may help explain why angiogenesis is primarily a feature of the hyperplastic rheumatoid synovium, even though VPF/VEGF was found in gout, osteoarthritis, and other types of synovial fluids.

It is not known, at present, what regulates the increased expression of VPF/VEGF by synovial macrophages in RA. Earlier studies indicated that oil-induced peritoneal macrophages, but not resident peritoneal or alveolar macrophages, expressed levels of VPF/VEGF mRNA detectable by Northern analysis or ISH (6). However, little is known about the regulation of VPF/VEGF expression in macrophages. A number of cytokines have been demonstrated in synovial fluids and tissue of RA patients, including TNF, TGF-β, and IL-1 (29–31); it is possible that one or more of these may have induced VPF/VEGF expression in synovial macrophages. It is not yet known whether VPF/VEGF can induce expression of such cytokines in macrophages or endothelial cells.

In summary, it appears that VPF/VEGF is synthesized and

secreted by macrophages in actively inflamed RA synovial tissue and that secreted VPF/VEGF binds to specific receptors on endothelial cells of small nearby blood vessels. In view of the fact that both KDR and flt-1 are class III tyrosine kinase receptors (32), it is probably relevant that phosphotyrosine, the product of activated tyrosine-kinase receptors, has recently been demonstrated in vascular endothelium of rheumatoid synovium (33). VPF/VEGF appears in some ways to resemble other proangiogenic cytokines that have been implicated in synovial angiogenesis (fibroblast growth factor, IL-8) (34, 35), but with the rather unique attributes of inducibility by hypoxia (36) (a feature of arthritic joint tissue), the ability to induce vascular permeability, and a restricted mitogenic activity for endothelial cells.

We thank Dr. Forst Brown and Leland Hall for providing tissue specimens, and Jane Hunt for expert technical assistance.

This work was supported by a Veterans Affairs Merit Review grant and a Hitchcock Foundation grant (to R. A. Fava) and by United States Public Health Service/National Institutes of Health grants CA-43947 (to D. R. Senger), and CA-50453 and CA-58845 (to H. F. Dvorak).

Address correspondence to Dr. Roy A. Fava, Department of Veterans Affairs, Medical and Regional Office Center, White River Junction, VT 05009.

Received for publication 6 December 1993 and in revised form 7 April 1994.

References

1. Senger, D.R., L. Van De Water, L.F. Brown, J.A. Nagy, K.-T. Yeo, T.-K. Yeo, B. Berse, R.W. Jackman, A.M. Dvorak, and H.F. Dvorak. 1993. Vascular premeability factor (VPF/VEGF) in tumor biology. *Cancer Metastasis Rev.* 12:303.
2. Senger, D.R., S.J. Galli, A.M. Dvorak, C.A. Peruzzi, V.S. Harvey, and H.F. Dvorak. 1983. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science (Wash. DC).* 219:983.
3. Senger, D.R., D.T. Connolly, L. Van De Water, J. Feder, and H.F. Dvorak. 1990. Purification and NH_2-terminal amino acid sequence of guinea pig tumor-secreted vascular permeability factor. *Cancer Res.* 50:1774.
4. Yeo, K.T., H.H. Wang, J.A. Nagy, T.M. Sioussat, S.R. Ledbetter, A.J. Hoogewerf, Y. Zhou, E.M. Masse, D.R. Senger, H.F. Dvorak, and T.K. Yeo. 1993. Vascular permeability factor (vascular endothelial growth factor) in guinea pig and human tumor and inflammatory effusions. *Cancer Res.* 53:2912.
5. Ferrara, N., K. Houck, L. Jakeman, and D.W. Leung. 1992. Molecular and biological properties of the vascular endothelial cell growth factor family of proteins. *Endocr. Rev.* 13:18.
6. Berse, B., L.F. Brown, L. Van De Water, H.F. Dvorak, and D.R. Senger. 1992. Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. *Mol. Biol. Cell.* 3:211.
7. Brown, L.F., B. Berse, K. Tognazzi, E.J. Manseau, L. Van De Water, D.R. Senger, H.F. Dvorak, and S. Rosen. 1992. Vascular permeability factor mRNA and protein expression in human kidney. *Kidney Int.* 42:1457.
8. Brown, L.F., K.-T. Yeo, B. Berse, T.-K. Yeo, D.R. Senger, H.F. Dvorak, and L. Van De Water. 1992. Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. *J. Exp. Med.* 176:1375.
9. Peters, K.G., C. De Vries, and L.T. Williams. 1993. Vascular endothelial growth factor receptor expression during embryogenesis and tissue repair suggests a role in endothelial differentiation and blood vessel growth. *Proc. Natl. Acad. Sci. USA.* 90:8915.
10. Dvorak, H.F., T.M. Sioussat, L.F. Brown, B. Berse, J.A. Nagy, A. Sotrel, E.J. Manseau, L. Van De Water, and D.R. Senger. 1991. Distribution of vascular permeability factor (vascular endothelial growth factor) in tumors: concentration in tumor blood vessels. *J. Exp. Med.* 174:1275.
11. Jin Kim, K., B. Li, J. Winer, M. Armanini, N. Gillett, H.S. Phillips, and N. Ferrara. 1993. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth *in vivo*. *Nature (Lond.).* 362:841.
12. Colville-Nash, P.R., and D.L. Scott. 1992. Angiogenesis and rheumatoid arthritis: pathologic and therapeutic implications. *Ann. Rheum. Dis.* 51:919.
13. Peacock, D.J., M.L. Banquerigo, and E. Brahn. 1992. Angiogenesis inhibition suppresses collagen arthritis. *J. Exp. Med.* 175:1135.
14. Yeo, K.T., T.M. Sioussat, J.D. Faix, D.R. Senger, and T.K. Yeo. 1992. Development of time-resolved immunofluorometric assay of vascular permeability factor. *Clin. Chem.* 38:71.
15. Olsen, N.J., L.F. Callahan, and T. Pincus. 1988. In vitro rheumatoid factor synthesis in patients taking second-line drugs for rheumatoid arthritis: independent associations with disease activity. *Arthritis Rheum.* 31:1090.
16. Olsen, N.J., G.P. Teal, and V. Strand. 1993. *In vivo* T cell depletion in rheumatoid arthritis is associated with increased *in vitro* IgM-rheumatoid factor synthesis. *Clin. Immunol. Immunopathol.* 67:124.
17. Brown, L.F., B. Berse, F. Tognazzi, E.J. Manseau, L. Van De Water, D. Senger, H.F. Dvorak, and S. Rosen. 1992. Vascular permeability factor mRNA and protein expression in human kidney. *Kidney Int.* 42:1457.
18. Tischer, E., R. Mitchell, T. Hartman, M. Silva, D. Gospodarowicz, J.C. Fiddes, and J.A. Abraham. 1991. The human

gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. *J. Biol. Chem.* 266:11947.

19. Houck, K.A., N. Ferrara, J. Winer, G. Cachianes, B. Li, and D.W. Leung. 1991. The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. *Mol. Endocrinol.* 5:1806.
20. Sioussat, T.M., H.F. Dvorak, T.A. Brock, and D.R. Senger. 1993. Inhibition of vascular permeability factor (vascular endothelial growth factor) with antipeptide antibodies. *Arch. Biochem. Biophys.* 301:15.
21. Hui, K.Y., E. Haber, and G.R. Matsueda. 1983. Monoclonal antibodies to a synthetic fibrin-like peptide bind to human fibrin but not fibrinogen. *Science (Wash. DC).* 222:1129.
22. Trentham, D.E., A.S. Townes, and A.H. Kang. 1977. Autoimmunity to type II collagen: an experimental model of arthritis. *J. Exp. Med.* 146:857.
23. Brock, T.A., H.F. Dvorak, and D.R. Senger. 1991. Tumor-secreted vascular permeability factor increases cytosolic Ca^{2+} and von Willebrand factor release in human endothelial cells. *Am. J. Pathol.* 138:213.
24. Pulford, K.A., E.M. Rigney, K.J. Micklem, M. Jones, W.P. Stross, K.C. Gatter, and D.Y. Mason. 1989. KP1: a new monoclonal antibody that detects a monocyte/macrophage associated antigen in routinely processed tissue sections. *J. Clin. Pathol.* 42:414.
25. Zacharski, L.R., F.E. Brown, V.A. Memoli, W. Kisiel, B.J. Kudryk, S.M. Rousseau, J.A. Hunt, C. Dunwiddie, and E.M. Nutt. 1992. Pathways of coagulation activation *in situ* in rheumatoid synovial tissue. *Clin. Immunol. Immunopathol.* 63:155.
26. Weinberg, J.B., A.A.M. Pippen, and C.S. Greenberg. 1991. Extravascular fibrin formation and dissolution in synovial tissue of patients with osteoarthritis and rheumatoid arthritis. *Arthritis Rheum.* 34:996.
27. Dvorak, H.F., V.S. Harvey, P. Estrella, L.P. Brown, J. McDonagh, and A.M. Dvorak. 1987. Fibrin containing gels induce angiogenesis. Implications for tumor stroma generation and wound healing. *Lab. Invest.* 57:673.
28. Brown, L.F., B. Berse, R.W. Jackman, K. Tognazzi, E.J. Manseau, D.R. Senger, and H.F. Dvorak. 1993. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. *Cancer Res.* 53:4727.
29. DiGiovine, F.S., G. Nuki, and G.W. Duff. 1988. Tumor necrosis factor in synovial exudates. *Ann. Rheum. Dis.* 47:768.
30. Fava, R., N. Olsen, J. Keski-Oja, H. Moses, and T. Pincus. 1989. Active and latent form of transforming growth factor β activity in synovial effusions. *J. Exp. Med.* 169:291.
31. Wahl, S.M., J.B. Allen, H.L. Wong, S.F. Dougherty, and L.R. Ellingsworth. 1990. Antagonistic and agonistic effects of transforming growth factor-β and IL-1 in rheumatoid synovium. *J. Immunol.* 145:2515.
32. Ullrich, A., and J. Schlessinger. 1990. Signal transduction by receptors with tyrosine kinase activity. *Cell.* 61:203.
33. Sano, H., K. Engleka, P. Mathern, T. Hla, L.J. Crofford, E.F. Remmers, C.L. Jelsema, E. Goldmuntz, T. Maciag, and R.L. Wilder. 1993. Coexpression of phosphotyrosine-containing proteins, platelet-derived growth factor-β, and fibroblast growth factor-1 in situ in synovial tissues of patients with rheumatoid arthritis and Lewis rats with adjuvant or streptococcal cell wall arthritis. *J. Clin. Invest.* 91:553.
34. Koch, A.E., P.J. Polverini, S.L. Kunkel, L.A. Harlow, L.A. DiPietro, V.M. Elner, S.G. Elner, and R.M. Strieter. 1992. Interleukin-8 as a macrophage-derived mediator of angiogenesis. *Science (Wash. DC).* 258:1798.
35. Sano, H., R. Forough, J.A. Maier, J.P. Case, A. Jackson, K. Engleka, T. Maciag, and R.L. Wilder. 1990. Detection of high levels of heparin binding growth factor-1 (acidic fibroblast growth factor) in inflammatory arthritic joints. *J. Cell Biol.* 110:1417.
36. Shweiki, D., A. Itin, D. Soffer, and E. Keshet. 1992. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature (Lond.).* 359:843.