# Angiogenesis-Inflammation Cross-Talk: Vascular Endothelial Growth Factor Is Secreted by Activated T Cells and Induces Th1 Polarization

**Felix Mor,*† Francisco J. Quintana,* and Irun R. Cohen[1,2]***

**Vascular endothelial growth factor (VEGF) and its receptors are critical in angiogenesis. The main player in the secretion and response to VEGF is the endothelial cell. We initiated this study to test whether T cells can secrete VEGF and are able to respond to it. Here we show that VEGF is secreted by T cells on stimulation by specific Ag or by IL-2 and by hypoxia; thus, activated T cells might enhance angiogenesis. Hypoxia also induced the expression in T cells of VEGFR2, suggesting that T cells might also respond to VEGF. Indeed, VEGF augmented IFN-$\gamma$ and inhibited IL-10 secretion by T cells responding to mitogen or Ag; thus, VEGF can enhance a Th1 phenotype. Encephalitogenic T cells stimulated in the presence of VEGF caused more severe and prolonged encephalomyelitis. Thus, T cells can play a role in angiogenesis by delivering VEGF to inflammatory sites, and VEGF can augment proinflammatory T cell differentiation.** ***The Journal of Immunology*****, 2004, 172: 4618–4623.**

The study of angiogenesis is a rapidly growing field. New blood vessel formation is critical in the growth and spread of malignant tumors (1) and in the development of microvascular complications of diabetes (2). Therapeutic induction of angiogenesis is being tested in the treatment of myocardial (3) and peripheral ischemic (4) syndromes. Angiogenesis is also important in inflammation. One of the pathological hallmarks of rheumatoid arthritis is the formation of pannus (5), an inflammatory connective tissue mass rich in blood vessels, apparently dependent on angiogenic factors (6, 7).

The endothelial cell has been considered to be the main cellular player in the secretion of angiogenic factors and in the response to them (8). Vascular endothelial growth factor (VEGF)[3] is a specific mitogen for vascular endothelial cells (9). VEGF was demonstrated by RT-PCR from various sources (10) including human peripheral blood leukocytes (11), which contain lymphocytes, monocytes, and other cells (12). In contrast, the expression of VEGF receptors has been reported to be limited to endothelial cells (8).

T cells are pivotal in the initiation and progression of inflammation (13). T cells have multiple effects on immune and nonimmune cells through their secretion of a large number of cytokines and chemokines (14). T cells homing to inflammatory sites are in intimate contact with endothelial cells (15) and could influence the angiogenesis associated with inflammation. Because inflammation involves new blood vessel formation (16), we tested whether T cells can deliver and respond to the angiogenic signal VEGF.

## Materials and Methods

### *Animals*

Inbred female Lewis rats were supplied by the animal breeding center of the Weizmann Institute of Science (Rehovot, Israel) under the supervision of Harlan Laboratories (Haslett, MI) and an animal welfare committee and were used at 2–3 mo of age.

### *Primers and Abs*

The primers for VEGF were: sense 5′-TGCACCCACGACAGAAGGGGA-3′ and antisense 5′-TCACCGCCTTGGCTTGTCACAT-3′ (product sizes, 497 bp for 120-aa isoform, 629 bp for 164-aa isoform, and 701 bp for 188-aa isoform). Primers for VEGFR1 (Flt-1) were sense 5′-CTTTCTCAAGTGCAGAGGGG-3′ and antisense 5′-AGGATTGTATTGGTCTGCCG-3′ (product size, 543 bp). Primers for VEGFR2 (KDR/Flk-1) were sense 5′-AAGCAAATGCTCAGCAGGTT-3′ and antisense 5′-TCTGTCTGGCTGTCATCTGG-3′ (product size, 283 bp). The Abs used were rabbit polyclonal anti-VEGF (sc-507), anti-Flt-1 (sc-316), anti-Flk-1 (sc-504) and anti-rabbit HRP-conjugated Ab, all from Santa Cruz Biotechnology (Santa Cruz, CA). The ELISA kit for murine VEGF was purchased from R&D Systems (Minneapolis, MN).

### *T cell lines and clones*

Ag-specific T cell lines were established from lymph node cells that had been stimulated with Ag (10 $\mu$g/ml) for 3 days in stimulation medium, as described (17). The T cell lines used were: anti-p277, reactive to peptide p277 of heat shock protein (HSP) 60 (18); BP10, a Lewis guinea pig myelin basic protein (MBP)-reactive T cell line (17); T cell clones 5, 9, 26, and 22 were derived from a line reactive to $\beta$-synuclein peptide $p_{93-111}$ (19); FISCHER, an MBP-reactive line derived from Fischer 344 rats; A2b, a clone derived from Lewis rats with adjuvant arthritis (20) reactive with peptide $p_{176-190}$ of mycobacterial HSP65 (21).

### *RT-PCR assay*

Total cellular RNA was isolated by the single-step method using the TRI REAGENT (Molecular Research Center, Cincinnati, OH). Five micrograms of total RNA were used for the reverse transcriptase reaction. cDNA was prepared, and PCR were performed as described (22).

### *Western blotting*

The protein concentration was determined using the Bio-Rad Dc protein assay, and 50 $\mu$g of protein were loaded per lane (Bio-Rad, Hercules, CA). After electrophoresis in 12% SDS gel in a minigel apparatus (Bio-Rad), the

*Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel; and †Department of Medicine A, Rabin Medical Center, Petach-Tiqva, affiliated with the Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

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[1] I.R.C. is the incumbent of the Mauerberger Chair in Immunology and the Director of the Center for the Study of Emerging Diseases.

[2] Address correspondence and reprint requests to Dr. Irun Cohen, Department of Immunology, The Weizmann Institute of Science, Rehovot, 76100, Israel. E-mail address: irun.cohen@weizmann.ac.il.

[3] Abbreviations used in this paper: VEGF, vascular endothelial growth factor; HSP, heat shock protein; MBP, myelin basic protein; EAE, experimental autoimmune encephalomyelitis.



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gels were electrotransferred to nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany). Immunoblotting was performed as described (23).

### *ELISA determination of rat VEGF*

The levels of VEGF in culture supernatants were measured by a VEGF ELISA kit according to the instructions of the manufacturer (R&D Systems). This assay, which was developed for mouse VEGF, can be used to measure rat VEGF, based on a 70% cross-species reactivity for the detecting Abs.

### *Hypoxia induction*

To induce hypoxia, activated T cells, which are metabolically activated and found to secrete VEGF, were incubated ($5 \times 10^6$/0.5 ml Eppendorf tube) in medium-filled closed tubes, at 37°C incubation for 1–4 h (24). After each hour, a tube was placed on ice and centrifuged, the pellet was used for RT-PCR or immunoblotting, and the supernatant was frozen at −80°C until examined for VEGF by ELISA.

### *Experimental autoimmune encephalomyelitis (EAE)*

Adoptive transfer of EAE was mediated by i.p. injection of $15 \times 10^6$ BP10-activated line cells (17). The BP10 line was activated by incubation for 3 days with MBP (10 $\mu$g/ml) in the absence or presence of recombinant human VEGF (500 ng/ml; Refs. 25 and 26). T cell blasts were isolated on LymphoPrep gradient (Nycomed, Oslo, Norway). Groups of female Lewis rats (five per group) were injected with the line cells and scored for clinical signs of EAE from day 3 postinjection. Clinical EAE was observed 3–12 days after T cell line injection. Clinical scoring was: +1, paralysis of tail; +1.5, paresis of posterior paws and ataxia; +2, paraplegia; +3, paralysis extending to thoracic spine; +4, a moribund state (17).

### *Cytokine ELISA*

Supernatants were collected after 3 days of stimulation of T cells with Con A or specific Ag. IL-10 and IFN-$\gamma$ in the culture supernatants were measured by ELISA using the PharMingen OPTEIA kit (PharMingen, San Diego, CA). PharMingen recombinant rat cytokines were used as standards for calibration curves. The results are expressed as picograms per milliliter. The lower limits of sensitivity for the experiments described were 30 pg/ml for both IL-10 and IFN-$\gamma$. Recombinant human IL-1$\alpha$ and IL-1$\beta$ and VEGF were obtained from The National Cancer Institute preclinical repository (Rockville, MD). The recombinant human VEGF was produced by R&D in Sf21 cells, and the protein was purified by sequential chromatography (>97% purity by SDS-PAGE, visualized by silver staining). Recombinant human VEGF is active in the rat (27, 28).

## Results

### *VEGF expression is induced by IL-2 in T cells*

To detect the expression in different rat tissues of the various VEGF isoforms by RT-PCR, we used oligonucleotide primers spanning the eight exons of the VEGF gene (29). Similar to previous reports (10), we detected expression in rat tissues of three isoforms, 120 aa, 164 aa, and 188 aa (not shown). To examine the effect of IL-2 on VEGF expression by T cells, we incubated a rat T cell line (anti-p277) with 100 U/ml rIL-2 for various times (after 24 h of IL-2 deprivation), in the absence of Ag and APCs, and probed cDNA was prepared from the T cells. Fig. 1*a* shows that two isoforms of VEGF (encoding 120 aa and 164 aa; Ref. 29) were induced in the T cell line by incubation with IL-2 for 120 min. Incubation of the T cells for 120 min with culture medium containing TCGFs (30) also induced the VEGF isoforms.

To detect VEGF at the protein level, we lysed an activated T cell line (BP10) and various other tissues and ran SDS-PAGE separation with 50 $\mu$g of protein per lane. A 21-kDa band representing VEGF164 (the isoform containing 164 aa, analogous to the human VEGF165) was detected in lysates of various rat tissues including T line cells (Fig. 1*b*). In several tissues (fat and kidney lysates; Fig. 1*b*), we detected a higher isoform corresponding in size to the 205-aa isoform of VEGF. A band of identical size (21 kDa) was detected by the Ab with purified recombinant human VEGF (not shown).

We also treated T line cells with 5, 10, 25, 50, or 100 U/ml IL-1$\alpha$, IL-1$\beta$, or IL-2 for 24 h. Similar to the result seen in RT-PCR, IL-2 induced (in a dose-dependent manner) the expression of VEGF164 at the protein level (Fig. 1*c*). In contrast, incubation with IL-1$\alpha$ or IL-1$\beta$ did not induce VEGF expression, although these cytokines stimulated proliferation of the T cells as tested by thymidine incorporation (not shown). Thus, T cell proliferation by itself does not appear to induce VEGF. We may conclude that the induction of VEGF by IL-2 is probably unrelated to its mitogenic effect on T cells.

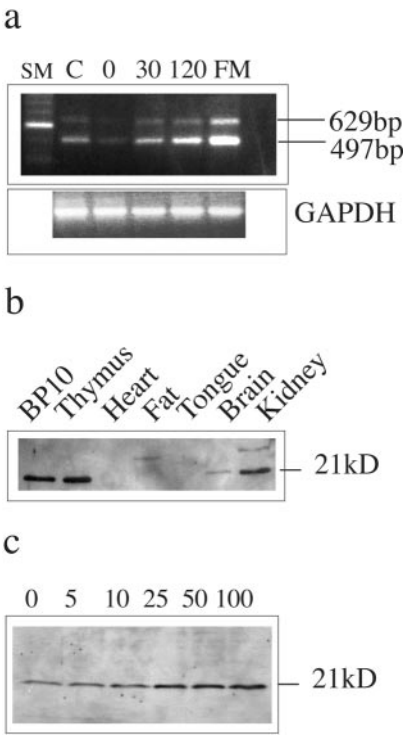


**FIGURE 1.** Induction of VEGF mRNA and protein by IL-2. *a*, PCR analysis of cDNA prepared from T cell line anti-p277 (18), which was starved from IL-2 for 24 h (indicated as 0), and incubated with 100 U/ml recombinant human IL-2 for 30 or 120 min (indicated as 30 and 120). FM, Incubation with full medium (containing T cell growth factors (TCGF) from supernatants of Con A-stimulated spleen cells; Ref. 30). SM, Size marker. PCR amplification was performed with primers spanning from exon 1 to exon 8. The two bands seen (497 and 629 bp) represent the two VEGF isoforms (120 and 164 aa). Control (C) cDNA was amplified with primers for GAPDH. *b*, Immunoblotting of various rat tissues with rabbit polyclonal Ab against VEGF. The 21-kDa band is seen in BP10 (a MBP-reactive T cell line (17), at the end of stimulation) and represents the 164-aa isoform. *c*, Induction of VEGF by graded concentrations of IL-2. Anti-p277 line cells were incubated for 24 h with 0, 5, 10, 25, 50, or 100 U/ml IL-2. Cells were collected, lysed, and tested by SDS-PAGE. Blotted membranes were immunostained with polyclonal rabbit anti-VEGF. A dose response to IL-2 was seen. With higher concentrations of IL-2, the 21-kDa VEGF band is induced.

### *VEGF is secreted by T cells*

We investigated whether T cells secrete VEGF and whether this secretion can be activated by specific Ag stimulation. We screened for the presence of VEGF in the medium of various T cell clones and lines that had been stimulated with specific Ag for 3 days. Fig. 2a shows that six of seven T cell lines and clones produced VEGF as detected by ELISA. Fig. 2*b* shows that increasing amounts of VEGF were secreted by two representative T cell lines during 4 days of Ag stimulation; the amount of VEGF in the culture supernatants increased progressively from day 1 to day 4 of Ag-induced stimulation in both the anti-p277 and the FISCHER lines. VEGF was also detected in the supernatant of Con A-activated spleen

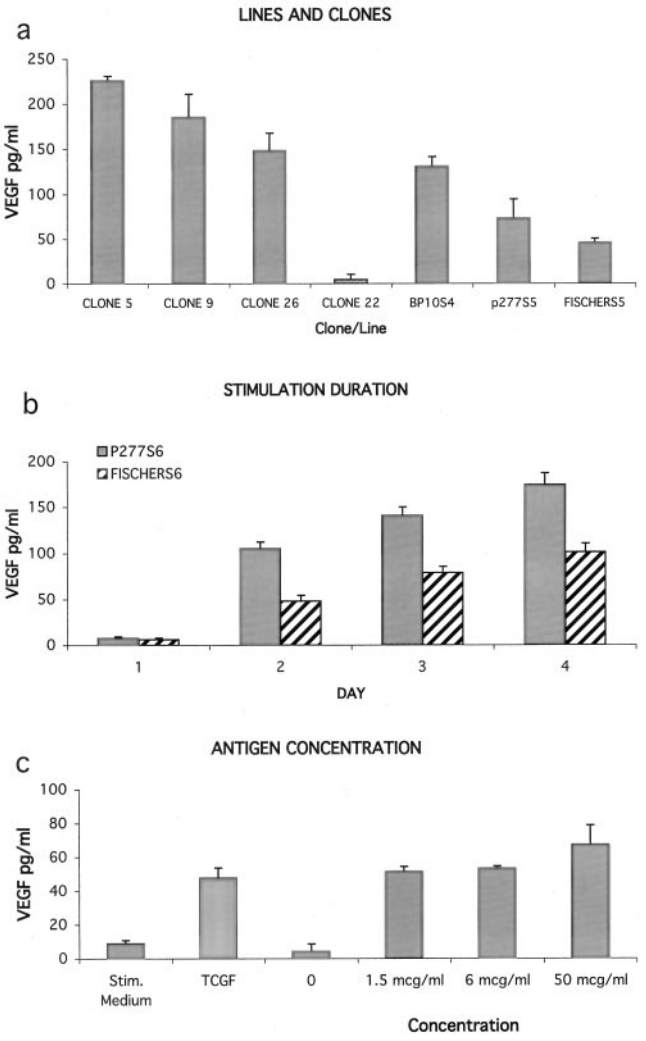


**FIGURE 2.** Secretion of VEGF by T cells. *a*, VEGF ELISA results of third-day activation supernatants of several T cell clones and lines. The clones were derived from a T cell line to $\beta$-synuclein (19). The FISCHER is a MBP-reactive T cell line derived from Fischer rats at the fifth in vitro stimulation (Stim.) (FISCHERS5). BP10S4 indicates BP10 line at the fourth stimulation, p277S5 is anti-p277 line at the fifth stimulation. *b*, Cumulative daily secretion of VEGF by Ag-specific T cell lines during T cell activation. Progressive increases in the amounts of VEGF are detected from day 1 to day 4. p277S6, Anti-p277 line at the sixth stimulation; FISCHERS6, FISCHER line at the sixth stimulation. *c*, Dose-dependent induction of VEGF secretion. The anti-p277 line was incubated for 3 days with the peptide concentrations indicated, and supernatants were tested by ELISA. TCGF, The second day supernatant of Con A-activated rat spleen cells (30).

cells (Fig. 2*c*; TCGF). This finding indicates that the ability to secrete VEGF is present in primary lymphocytes and not only in T cell lines and clones.

*Induction of VEGF and VEGFR2 mRNA and protein by hypoxia*

Hypoxia is a physiological stimulus for VEGF induction in endothelial cells (31). To examine whether hypoxia might also induce VEGF expression in T cells, line T cells 1 day after Ag stimulation (when the cells are highly active and divide in response to IL-2) were incubated in closed Eppendorf tubes for 1–3 h at 37°C, deprived of oxygen (24). The hypoxia resulted in a time-dependent induction of VEGF and VEGFR2 (KDR/Flk-1) mRNA in the T cells (Fig. 3*a*). In contrast to VEGFR2, mRNA for VEGF receptor 1 (Flt-1), which we readily detected in other rat tissues by RT-

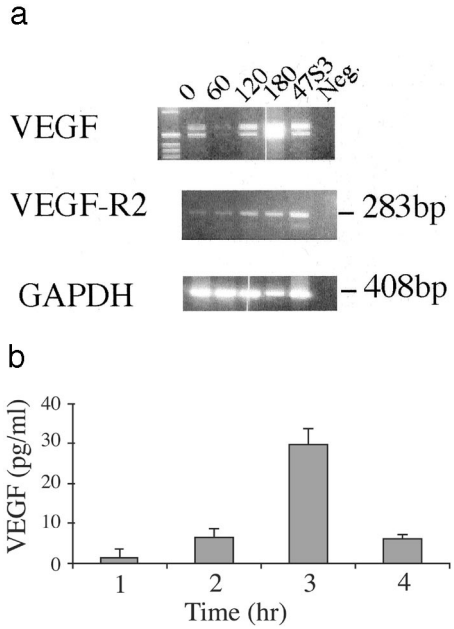


**FIGURE 3.** Induction of VEGF and VEGFR2 by hypoxia. Anti-p277 line cells at the beginning (day 1) of the propagation phase were incubated in closed Eppendorf tubes for the times indicated; cDNA was prepared and tested with primers for VEGF and VEGFR2. Both VEGF and VEGFR2 were induced by hypoxia and levels of PCR products peaked at 180 min. 47S3, cDNA from the T cell line reactive to $\beta$-synuclein peptide aa 93–111 (19). Control cDNA was amplified with primers for GAPDH. *b*, Induction of VEGF secretion by hypoxia. T cell line (anti-p277) was incubated at the end of Ag stimulation for 1–4 h in closed Eppendorf tubes and supernatants were tested by ELISA.

PCR, was undetectable in the T cells (data not shown). To test the effects of hypoxia on VEGF secretion, Ag-stimulated line cells were incubated for 1–4 h in the absence of oxygen, and the supernatants were examined for the VEGF protein by ELISA. VEGF in the medium was found to increase progressively until the third hour and decreased at the fourth hour (Fig. 3*b*). The decrease in the fourth hour may indicate binding and internalization of VEGF or degradation by enzymes secreted by the hypoxic T cells. Immunoblot analysis of T cell lysates demonstrated the production of VEGFR2 protein (not shown).

*VEGF enhances a Th1 cytokine response profile*

The results presented above show that T cells can synthesize and secrete VEGF and express VEGFR2. Because VEGFR2 is the receptor known to transduce VEGF signaling in endothelial cells (8), we tested whether VEGF could affect T lymphocytes. Incubation of T cells with VEGF did not induce proliferation. Because VEGF prevents apoptosis in endothelial cells (32), we tested the ability of VEGF to prevent dexamethasone-induced apoptosis (18) in both resting and activated T cells. The results of these experiments failed to show any antiapoptotic effect of VEGF on T cells (not shown).

However, we found that T cells responded to VEGF by modifying their cytokine secretion. We activated naive lymph node cells by various concentrations of the T cell mitogen Con A with or without VEGF (500 ng/ml; Refs. 25 and 26). Fig. 4 shows that the presence of VEGF induced a modest elevation of IFN-$\gamma$ secretion and a decrease in IL-10 secretion at various concentrations of Con A. An increase in IFN-$\gamma$ and a decrease in IL-10 is consistent with polarization toward Th1 differentiation (Fig. 4, *left*). VEGF also influenced the cytokine profile of primed LNC responding to a specific Ag. We obtained draining popliteal lymphocytes from

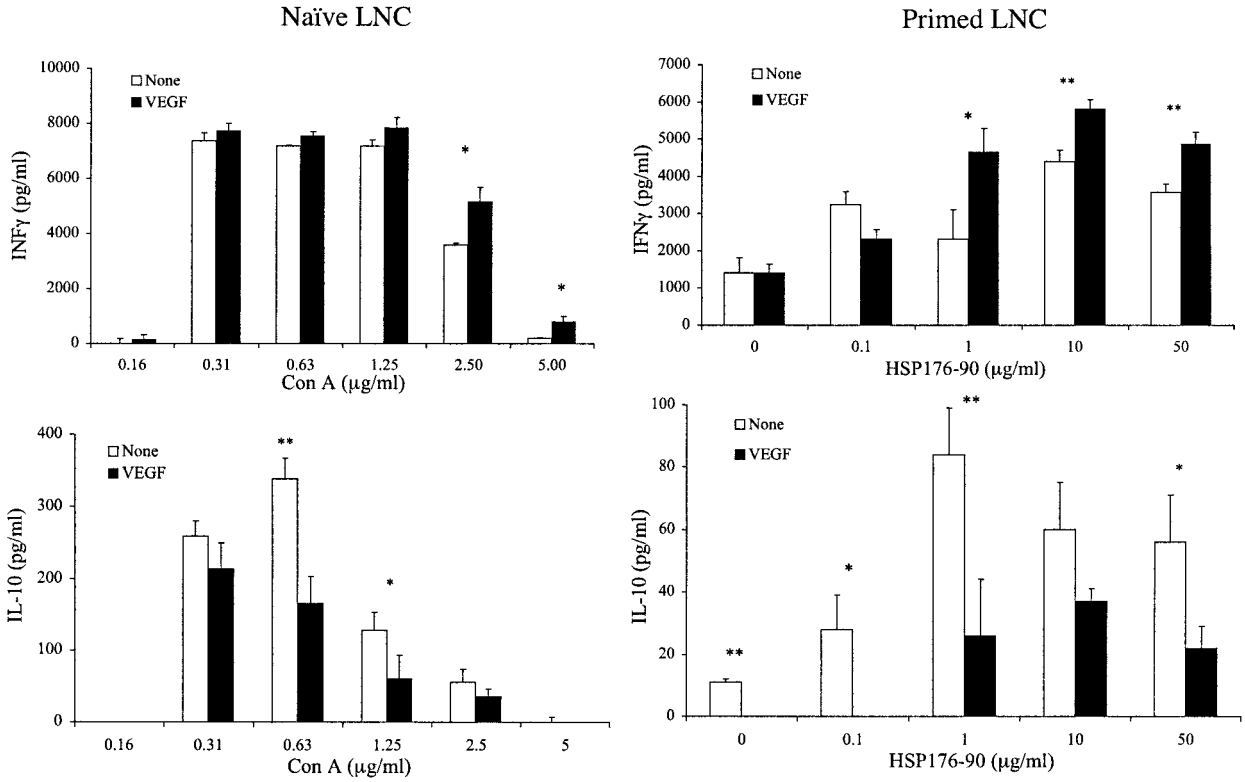


**FIGURE 4.** VEGF augments Th1 response in lymph node cells (LNC). *Left,* ELISA results of IFN-γ and IL-10 in supernatants of naive lymph node cells stimulated with graded concentrations of Con A in the presence or absence of VEGF. *Right,* ELISA of lymph node cells from CFA-immunized rats (primed LNC). Levels of significance were calculated using *t* test, InStat 2.01 (GraphPad software, CA); ∗, $0.005 < p < 0.05$; ∗∗, $p < 0.005$.

rats that had recovered from adjuvant arthritis induced 60 days earlier (33), a time point when the rat T cells express also Th2 cytokines in response to target Ags. We stimulated responding T cells with the HSP65 target peptide p176–190 (21). Fig. 4 (*right*) shows that here too, the presence of VEGF resulted in enhancement of IFN-γ at 1, 10, and 50 μg/ml Ag and inhibition of IL-10 secretion by the responding T cells at 0.1, 1, and 50 μg/ml. Fig. 5 (*left*) shows that BP10, a MBP-specific T cell line, also secreted more IFN-γ and less IL-10 in the presence of VEGF.

To rule out the possibility that the VEGF might have influenced T cell cytokine secretion indirectly by way of APCs or other non-T cells present in the above cultures, we tested the effects of VEGF on a T cell clone (A2b; Ref. 20) that has high expression of MHC class II and proliferates to its specific peptide in the absence of exogenous APCs (21); autopresentation by T cells without APC has been described in human (34) and rat (21) T cells. In this experiment, any effect of VEGF on cytokine production had to be attributed directly to the responding T cells themselves. Here too, the presence of VEGF during stimulation of clone A2b alone resulted in a marked increase in IFN-γ and a decrease in IL-10 secretion (Fig. 5, *right*).

### *Enhancement of T cell-mediated EAE by VEGF*

EAE is mediated by Th1 T cells reactive to myelin Ags (35). To examine the functional effect of VEGF on T cell mediated inflammation, we stimulated an encephalitogenic T cell line (BP10) with or without VEGF (500 ng/ml; 25 and 26) and injected the activated line cells into naive rats. The VEGF-treated T cells mediated the earlier onset of a more severe and prolonged EAE compared with the T cells stimulated without VEGF (Fig. 6). Thus, VEGF augmented adoptively transferred EAE disease; the enhanced Th1 profile detected in vitro was accompanied by a significant enhancement of the disease in vivo.

## Discussion

Previous work has demonstrated the expression of VEGF by peripheral blood lymphocytes (11, 36); but, in addition to T cells, such populations also contain other cells such as monocytes and thrombocytes that might secrete VEGF (12). Other investigators have documented the expression of VEGF by malignant (37) or virus-infected T cells (38, 39). Malignant B cells from patients with chronic lymphatic leukemia (37) and T cells infected with HIV (38) and human T cell leukemia virus type 1 (39) were reported to express VEGF. Basic fibroblast growth factor has been reported to be produced by T cells (40).

The present results demonstrate that both primary T cells stimulated by Con A and primed T cells, T cell lines, and clones responding to specific Ags synthesize and secrete VEGF (Figs. 1 and 2). Secondly, VEGF secretion can be activated by the concentration and duration of stimulation by specific Ag (Fig. 2) or by IL-2 (Fig. 1). Third, both VEGF and VEGFR2 are induced in T cells by hypoxia (Fig. 3). Fourth, VEGF was found to augment secretion of IFN-γ and inhibit secretion of IL-10 in primary T cells and in T cell lines (Figs. 4 and 5). Finally, incubation of a pathogenic T line with VEGF resulted in more severe encephalomyelitis, indicating that the in vitro effect on cytokine secretion was significant in modulation of the in vivo effects of the line (Fig. 6).

The finding that T cells synthesize and secrete VEGF has implications for both the physiology and pathophysiology of inflammation. Aberrant angiogenesis is a characteristic of abnormal inflammation in autoimmune diseases such as rheumatoid arthritis (5), and angiogenesis is a damaging factor in retinitis (41). It is conceivable that the T

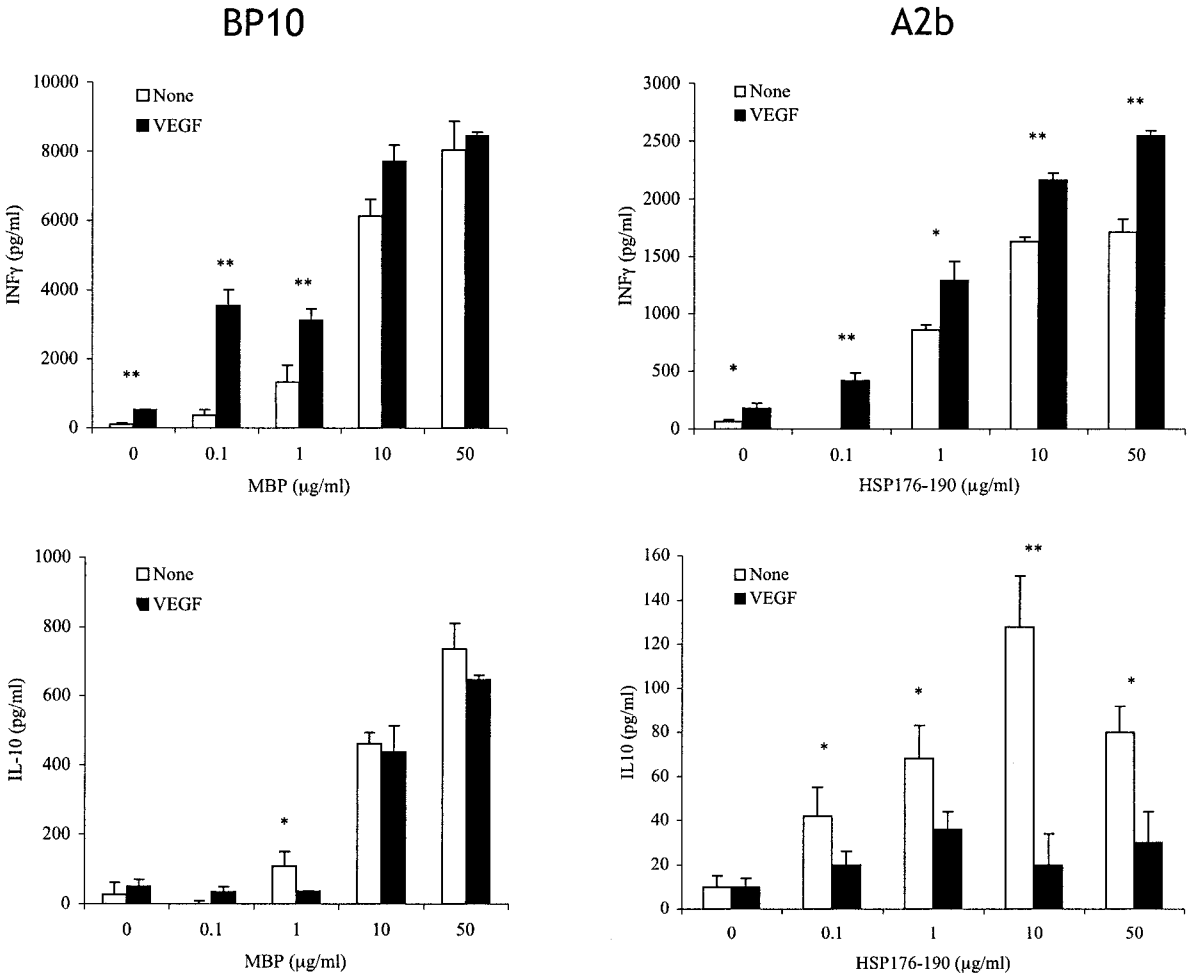


**FIGURE 5.** VEGF augments a Th1 response in T cell lines. ELISA results of cytokine measurements of supernatants of Ag stimulation of line BP10 in the presence of APCs (BP10, *left*). Cytokine measurements of clone A2b stimulated with peptide $p_{176-190}$ of HSP65 in the absence of APC (*right*). Levels of significance in *t* test are as shown in the legend to Fig. 4.

cells infiltrating autoimmune lesions (42, 43) contribute to pathogenic angiogenesis by secreting VEGF when they respond to target self-Ags or to the IL-2 produced by other activated T cells.

Physiological angiogenesis too could benefit from VEGF produced by activated T cells. VEGF produced by T cells could activate endothelial cells at sites of T cell activation such as draining lymph nodes. Immune responses are marked by increases in lymph node size and vascularity (44). The present results indicate that activated T cells producing VEFG could contribute to the physiology of lymph node vascularity. Thus, the degree of new blood vessel formation could be fine-tuned by the site and degree of T cell activation.

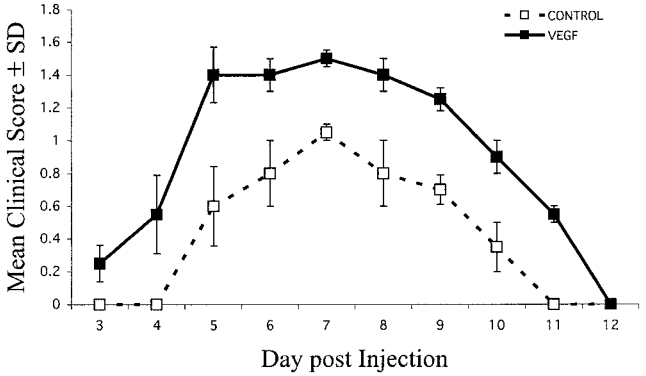


**FIGURE 6.** Effects of VEGF on EAE. Administration of BP10 line cells to rats after incubation with VEGF during Ag activation results in earlier and more severe disease. At each time point, disease scores differed significantly between the groups (Mann-Whitney *U* test; InStat software, $p = 0.05$ day 5; $p = 0.03$ day 6; $p = 0.008$ days 7 and 9; $p = 0.01$ day 8).

We and others have shown that activated T cells can serve as vehicles for neurotrophic factors (45), and it is not unlikely that T cell neurotrophins could play a role in the positive contribution of autoimmune T cells to neuroprotection after trauma to the CNS (46). The present findings that activated T cells produce VEGF suggest that activated T cells might also deliver needed angiogenic factors to sites of injury and so promote healing.

Inflammation has been defined as a process induced by damage that normally leads to healing (47). Thus, the role of T cells in inflammation can include molecular signals that can enhance tissue healing, not only tissue destruction (48, 49). The homing of T cells to sites of inflammation provides a mobile source of VEGF that can be controlled by the degree of T cell activation. Moreover, hypoxia associated with tissue damage would lead to the production angiogeneic factors including VEGF at sites of damage; the VEGF would, as we show here, push T cells visiting the site into a proinflammatory Th1 mode and amplify inflammation. VEGF is an important signal in the dialog between the tissues and the immune system (48). It would be of interest to test whether the blood

vessels at inflammatory sites are indeed newly formed (50, 51) and whether inhibition of VEGF signaling by small molecules (52) or Abs (16) could affect the evolution of the inflammatory process.

## References


1. Folkman, J. 1992. The role of angiogenesis in tumor growth. *Semin. Cancer Biol. 3:65*.
2. Pe'er, J., R. Folberg, A. Itin, H. Gnessin, I. Hemo, and E. Keshet. 1996. Upregulated expression of vascular endothelial growth factor in proliferative diabetic retinopathy. *Br. J. Ophthalmol. 80:241*.
3. Isner, J. M. 2002. Myocardial gene therapy. *Nature 415:234*.
4. Isner, J. M. 1999. Manipulating angiogenesis against vascular disease. *Hosp. Pract. (Off. Ed.). 34:69*.
5. Hau, M., C. Kneitz, H. P. Tony, M. Keberle, R. Jahns, and M. Jenett. 2002. High resolution ultrasound detects a decrease in pannus vascularisation of small finger joints in patients with rheumatoid arthritis receiving treatment with soluble tumour necrosis factor $\alpha$ receptor (etanercept). *Ann. Rheum. Dis. 61:55*.
6. Afuwape, A. O., S. Kiriakidis, and E. M. Paleolog. 2002. The role of the angiogenic molecule VEGF in the pathogenesis of rheumatoid arthritis. *Histol. Histopathol. 17:961*.
7. Kasama, T., F. Shiozawa, K. Kobayashi, N. Yajima, M. Hanyuda, H. T. Takeuchi, Y. Mori, M. Negishi, H. Ide, and M. Adachi. 2001. Vascular endothelial growth factor expression by activated synovial leukocytes in rheumatoid arthritis: critical involvement of the interaction with synovial fibroblasts. *Arthritis Rheum. 44:2512*.
8. Ferrara, N., and T. Davis-Smyth. 1997. The biology of vascular endothelial growth factor. *Endocr. Rev. 18:4*.
9. Connolly, D. T. 1991. Vascular permeability factor: a unique regulator of blood vessel function. *J. Cell. Biochem. 47:219*.
10. Wellmann, S., T. Taube, K. Paal, V. E. H. Graf, W. Geilen, G. Seifert, C. Eckert, G. Henze, and K. Seeger. 2001. Specific reverse transcription-PCR quantification of vascular endothelial growth factor (VEGF) splice variants by LightCycler technology. *Clin. Chem. 47:654*.
11. Freeman, M. R., F. X. Schneck, M. L. Gagnon, C. Corless, S. Soker, K. Niknejad, G. E. Peoples, and M. Klagsbrun. 1995. Peripheral blood T lymphocytes and lymphocytes infiltrating human cancers express vascular endothelial growth factor: a potential role for T cells in angiogenesis. *Cancer Res. 55:4140*.
12. Gunsilius, E., A. Petzer, G. Stockhammer, W. Nussbaumer, P. Schumacher, J. Clausen, and G. Gastl. 2000. Thrombocytes are the major source for soluble vascular endothelial growth factor in peripheral blood. *Oncology 58:169*.
13. Anderton, S. M., and D. C. Wraith. 2002. Selection and fine-tuning of the autoimmune T-cell repertoire. *Nat. Rev. Immunol. 2:487*.
14. Glimcher, L. H., and K. M. Murphy. 2000. Lineage commitment in the immune system: the T helper lymphocyte grows up. *Genes Dev. 14:1693*.
15. Yellin, M. J., J. Brett, D. Baum, A. Matsushima, M. Szabolcs, D. Stern, and L. Chess. 1995. Functional interactions of T cells with endothelial cells: the role of CD40L-CD40-mediated signals. *J. Exp. Med. 182:1857*.
16. Luttun, A., M. Tjwa, L. Moons, Y. Wu, A. Angelillo-Scherrer, F. Liao, J. A. Nagy, A. Hooper, J. Priller, et al. 2002. Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-*Flt*1. *Nat. Med. 8:831*.
17. Mor, F., and I. R. Cohen. 1993. Shifts in the epitopes of myelin basic protein recognized by Lewis rat T cells before, during, and after the induction of experimental autoimmune encephalomyelitis. *J. Clin. Invest. 92:2199*.
18. Mor, F., and I. R. Cohen. 1996. IL-2 rescues antigen-specific T cells from radiation or dexamethasone- induced apoptosis: correlation with induction of Bcl-2. *J. Immunol. 156:515*.
19. Mor, F., F. Quintana, A. Mimran, and I. R. Cohen. 2003. Autoimmune encephalomyelitis and uveitis induced by T cell immunity to self $\beta$-synuclein. *J. Immunol. 170:628*.
20. Holoshitz, J., A. Matitiau, and I. R. Cohen. 1984. Arthritis induced in rats by cloned T lymphocytes responsive to mycobacteria but not to collagen type II. *J. Clin. Invest. 73:211*.
21. Taams, L. S., W. van Eden, and M. H. Wauben. 1999. Antigen presentation by T cells versus professional antigen-presenting cells (APC): differential consequences for T cell activation and subsequent T cell-APC interactions. *Eur. J. Immunol. 29:1543*.
22. Mor, F., B. Reizis, I. R. Cohen, and L. Steinman. 1996. IL-2 and TNF receptors as targets of regulatory T-T interactions: isolation and characterization of cytokine receptor-reactive T cell lines in the Lewis rat. *J. Immunol. 157:4855*.
23. Mor, F., A. Weinberger, and I. R. Cohen. 2002. Identification of $\alpha$-tropomyosin as a target self-antigen in Behçet's syndrome. *Eur. J. Immunol. 32:356*.
24. Ng, C. E., C. J. Koch, and W. R. Inch. 1980. Buoyant density of EMT6 fibrosarcoma cells: time course of the density changes after growth in hypoxia. *Cell Biophys. 2:153*.
25. Guedez, L., A. M. Rivera, R. Salloum, M. L. Miller, J. J. Diegmueller, P. M. Bungay, and W. G. Stelter-Stevenson. 2003. Quantitative assessment of angiogenic responses by the directed *in vivo* angiogenesis assay. *Am. J. Pathol. 162:1431*.
26. Cohen, A. W., J. M. Carbajal, and R. C. Schaeffer, Jr. 1999. VEGF stimulates tyrosine phosphorylation of $\beta$-catenin and small-pore endothelial barrier dysfunction. *Am. J. Physiol. 277:H2038*.
27. Deodato, B., N. Arsic, L. Zentilin, M. Galeano, D. Santoro, V. Torre, D. Altavilla, D. Valdembri, F. Bussolino, F. Squadrito, and M. Giacca. 2002. Recombinant AAV vector encoding human VEGF165 enhances wound healing. *Gene Ther. 9:777*.
28. Zhu, W. H., A. MacIntyre, and R. F. Nicosia. 2002. Regulation of angiogenesis by vascular endothelial growth factor and angiopoietin-1 in the rat aorta model: distinct temporal patterns of intracellular signaling correlate with induction of angiogenic sprouting. *Am. J. Pathol. 161:823*.
29. Burchardt, T., M. Burchardt, M. W. Chen, R. Buttyan, A. de la Taille, A. Shabsigh, and R. Shabsigh. 1999. Expression of VEGF splice variants 144/145 and 205/206 in adult male tissues. *IUBMB Life 48:405*.
30. Mor, F., and I. R. Cohen. 2002. Propagation of Lewis rat encephalitogenic T cell lines: T-cell-growth factor is superior to recombinant IL-2. *J. Neuroimmunol. 123:76*.
31. Shweiki, D., A. Itin, D. Soffer, and E. Keshet. 1992. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature 359:843*.
32. Gerber, H. P., A. McMurtrey, J. Kowalski, M. Yan, B. A. Keyt, V. Dixit, and N. Ferrara. 1998. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3′-kinase/Akt signal transduction pathway: requirement for Flk-1/KDR activation. *J. Biol. Chem. 273:30336*.
33. Quintana, F. J., P. Carmi, F. Mor, and I. R. Cohen. 2002. Inhibition of adjuvant arthritis by a DNA vaccine encoding human heat shock protein 60. *J. Immunol. 169:3422*.
34. LaSalle, J. M., F. Toneguzzo, M. Saadeh, D. E. Golan, R. Taber, and D. A. Hafler. 1993. T-cell presentation of antigen requires cell-to-cell contact for proliferation and anergy induction. Differential MHC requirements for superantigen and autoantigen. *J. Immunol. 151:649*.
35. Waisman, A., P. J. Ruiz, D. L. Hirschberg, A. Gelman, J. R. Oksenberg, S. Brocke, F. Mor, I. R. Cohen, and L. Steinman. 1996. Suppressive vaccination with DNA encoding a variable region gene of the T-cell receptor prevents autoimmune encephalomyelitis and activates Th2 immunity. *Nat. Med. 2:899*.
36. Iijima, K., N. Yoshikawa, and H. Nakamura. 1996. Activation-induced expression of vascular permeability factor by human peripheral T cells: a nonradioisotopic semiquantitative reverse transcription-polymerase chain reaction assay. *J. Immunol. Methods 196:199*.
37. Chen, H., A. T. Treweeke, D. C. West, K. J. Till, J. C. Cawley, M. Zuzel, and C. H. Toh. 2000. In vitro and in vivo production of vascular endothelial growth factor by chronic lymphocytic leukemia cells. *Blood 96:3181*.
38. Ascherl, G., C. Hohenadl, O. Schatz, E. Shumay, J. Bogner, L. Eckhart, E. Tschachler, P. Monini, B. Ensoli, and M. Sturzl. 1999. Infection with human immunodeficiency virus-1 increases expression of vascular endothelial cell growth factor in T cells: implications for acquired immunodeficiency syndrome-associated vasculopathy. *Blood 93:4232*.
39. El-Sabban, M. E., R. A. Merhi, H. A. Haidar, B. Arnulf, H. Khoury, J. Basbous, J. Nijmeh, H. de The, O. Hermine, and A. Bazarbachi. 2002. Human T-cell lymphotropic virus type 1-transformed cells induce angiogenesis and establish functional gap junctions with endothelial cells. *Blood 99:3383*.
40. Blotnick, S., G. E. Peoples, M. R. Freeman, T. J. Eberlein, and M. Klagsbrun. 1994. T lymphocytes synthesize and export heparin-binding epidermal growth factor-like growth factor and basic fibroblast growth factor, mitogens for vascular cells and fibroblasts: differential production and release by $CD4^+$ and $CD8^+$ T cells. *Proc. Natl. Acad. Sci. USA 91:2890*.
41. Vinores, S. A., C. C. Chan, M. A. Vinores, D. M. Matteson, Y. S. Chen, D. A. Klein, A. Shi, H. Ozaki, and P. A. Campochiaro. 1998. Increased vascular endothelial growth factor (VEGF) and transforming growth factor $\beta$ (TGF$\beta$) in experimental autoimmune uveoretinitis: upregulation of VEGF without neovascularization. *J. Neuroimmunol. 89:43*.
42. Steinman, L. 1996. A few autoreactive cells in an autoimmune infiltrate control a vast population of nonspecific cells: a tale of smart bombs and the infantry. *Proc. Natl. Acad. Sci. USA 93:2253*.
43. Flugel, A., T. Berkowicz, T. Ritter, M. Labeur, D. E. Jenne, Z. Li, J. W. Ellwart, M. Willem, H. Lassmann, and H. Wekerle. 2001. Migratory activity and functional changes of green fluorescent effector cells before and during experimental autoimmune encephalomyelitis. *Immunity 14:547*.
44. Campbell, I. K., K. O'Donnell, K. E. Lawlor, and I. P. Wicks. 2001. Severe inflammatory arthritis and lymphadenopathy in the absence of TNF. *J. Clin. Invest. 107:1519*.
45. Moalem, G., A. Gdalyahu, Y. Shani, U. Otten, P. Lazarovici, I. R. Cohen, and M. Schwartz. 2000. Production of neurotrophins by activated T cells: implications for neuroprotective autoimmunity. *J. Autoimmun. 15:331*.
46. Schwartz, M., and I. R. Cohen. 2000. Autoimmunity can benefit self-maintenance. *Immunol. Today 21:265*.
47. Florey, H. 1970. *General Pathology*. Lloyd-Luke Medical Books, London.
48. Cohen, I. R. 2000. Discrimination and dialogue in the immune system. *Semin. Immunol. 12:215*.
49. Cohen, I. R. 2000. *Tending Adam's Garden: Evolving the Cognitive Immune Self*. Academic Press, London.
50. Blankesteijn, W. M., M. E. van Gijn, Y. P. Essers-Janssen, M. J. Daemen, and J. F. Smits. 2000. $\beta$-catenin, an inducer of uncontrolled cell proliferation and migration in malignancies, is localized in the cytoplasm of vascular endothelium during neovascularization after myocardial infarction. *Am. J. Pathol. 157:877*.
51. Wikstrom, P., I. F. Lissbrant, P. Stattin, L. Egevad, and A. Bergh. 2002. Endoglin (CD105) is expressed on immature blood vessels and is a marker for survival in prostate cancer. *Prostate 51:268*.
52. Sukbuntherng, J., G. Cropp, A. Hannah, G. S. Wagner, L. K. Shawver, and L. Antonian. 2001. Pharmacokinetics and interspecies scaling of a novel VEGF receptor inhibitor, SU5416. *J. Pharm. Pharmacol. 53:1629*.