Vascular Endothelial Growth Factor Effects on Nuclear Factor-κB Activation in Hematopoietic Progenitor Cells

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ABSTRACT

Vascular endothelial growth factor (VEGF) inhibits the activation of transcription factor nuclear factor-κB (NF-κB) in hematopoietic progenitor cells (HPCs), and this is associated with alterations in the development of multiple lineages of hematopoietic cells and defective immune induction in tumor-bearing animals. Antibodies to VEGF have been shown to abrogate this effect. The mechanism by which VEGF antagonizes the maturation of dendritic cells, the professional antigen-presenting cells, in vitro and in vivo, and dramatically affects the maturation of other hematopoietic cells (11, 12).

Ineffective antigen presentation is one of the critical factors involved in tumor evasion from immune system control and plays an important role in immunological abnormalities observed in other conditions associated with hyperproduction of VEGF. Therefore, the effects of VEGF on hematopoiesis could be an important factor in the pathophysiology of these diseases as well. The significant molecular consequences of VEGF-mediated activation by VEGF in HPCs are unclear. One possible mechanism is suggested by our recent demonstration that the effects of VEGF are closely associated with the inhibition of TNF-α-inducible activation of the transcription factor NF-κB in HPCs (10). This DNA-binding transcription factor is composed of five subunits: p65 (RelA), p50, p52, c-Rel, and RelB (13). These different family members can associate in various homo- or heterodimers through a highly conserved NH2-terminal Rel homology domain. In the cytoplasm of quiescent cells, they are associated with inhibitory molecules of the IκB family. Cell activation by various stimuli, including TNF-α, lipo polysaccharide, interleukin 1, CD40, and others, result in serine phosphorylation and degradation of IκB with subsequent nuclear translocation and specific DNA binding of NF-κB dimers (14). It has also been suggested that tyrosine phosphorylation of IκBα can activate NF-κB without degradation (15). NF-κB plays a critical role in the differentiation of dendritic cells and B cells, and its involvement in T-cell development has been also demonstrated recently (16–21). These data suggest that inhibition of NF-κB could be responsible for the observed effects of VEGF on HPCs. However, it is unclear how VEGF might affect NF-κB activation. It is also unclear whether this mechanism is restricted to HPCs or might apply to other tissue targets of VEGF action. In this study, we investigated the mechanism of VEGF action on both HPCs and vascular endothelial cells. We showed that VEGF inhibited NF-κB activation in HPCs, but not in endothelial cells. This effect was associated with the ability of VEGF to induce translocation in HPCs of RelB-containing NF-κB complexes, but that RelB is not required for VEGF to inhibit NF-κB activation by TNF-α. Instead, VEGF works through a mechanism independent of VEGFR kinase to inhibit the activation of IKK, thus preventing serine phosphorylation and proteolytic degradation of the NF-κB inhibitory subunits IκBα and IκBε in HPCs.

INTRODUCTION

VEGF is a M<sub>f</sub> 34,000–43,000 protein that stimulates endothelial cell growth and plays a critical role in angiogenesis (1–3). VEGF production is dramatically increased in many pathological conditions, especially local hypoxia (4–6). VEGF plays an especially important role in cancer. Almost all tumor cells produce VEGF, and elevated levels are frequently detected in sera from cancer patients. VEGF production is closely associated with a poor prognosis (6–9). We have demonstrated previously that, in addition to its effects on angiogenesis, VEGF also binds to hematopoietic progenitor cells through the VEGF receptor 1 (Flt1; Ref. 10). This results in inhibition of the maturation of dendritic cells, the professional antigen-presenting cells, and prevents serine phosphorylation and proteolytic degradation of IκB with subsequent nuclear translocation and DNA binding of the complexes containing RelB. These results were confirmed by immunofluorescence confocal microscopy. VEGF effectively blocked TNF-α-induced NF-κB activation in HPCs from RelB<sup>−/−</sup> mice, however, similar to the effect observed in HPCs obtained from RelB<sup>+/−</sup> and RelB<sup>+/+</sup> mice. This suggests that RelB is not required for VEGF to inhibit NF-κB activation. However, although TNF-α induced rapid activation of IκB kinase (IKK) as expected, this activity was substantially reduced in the presence of VEGF. This decreased IKK activation correlated with the inhibition of IκBα phosphorylation and degradation of IκBα in HPCs. VEGF alone, however, did not have any effect on phosphorylation of IκBα or degradation of IκBα and other inhibitory molecules IκBγ, IκBε, or Rel-3. S5416, a potent inhibitor of the VEGF receptor 1 (VEGFR1) and VEGFR2 receptor tyrosine kinases, did not abolish the inhibitory effect of VEGF, indicating that the VEGF effect is mediated by a mechanism unrelated to VEGFR1 or VEGFR2 tyrosine kinase activity. Thus, VEGF appears to inhibit TNF-α-induced NF-κB activation by VEGFR kinase-independent inhibition of IKK. Therapeutic strategies aimed at overcoming VEGF-mediated defects in immune induction in tumor-bearing hosts will need to target this kinase-independent pathway.

MATERIALS AND METHODS

Mice. Female BALB/c and C57BL/6 mice, 6–8 weeks of age, were purchased from Harlan, Inc. (Indianapolis, IN) and were housed in specific pathogen-free units of the Division of Animal Care at Vanderbilt University Medical Center. RelB-deficient mice were obtained from Dr. D. Lo (The Scripps Research Institute, La Jolla, CA). These mice were generated on an inbred C57BL/6J background and were described in detail elsewhere (19).

Cell Lines and Reagents. RMCs were prepared from male Wistar rats as described (22). Cells were maintained in DMEM (Life Technologies, Inc., Gaithersburg, MD) and were used between passages 5 and 10. HMEC-1 cells were grown in MCDB131 medium (Sigma Chemical Co., St. Louis, MO).

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4 The abbreviations used: VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; HPC, hematopoietic progenitor cell; bHPC, human HPC; mHPC, mouse HPC; HMEC, human dermal microvascular endothelial cell; HUVEC, human umbilical vein endothelial cell; IKK, IκB kinase; NIK, IκB-inducing kinase; EMSA, electrophoretic mobility shift assay; GM-CSF, granulocyte/macrophage-stimulating factor; RMC, rat glomerular mesangial cell; GST, glutathione S-transferase.
containing 15% fetal bovine serum (Hyclone Laboratories, Logan UT), 10 ng/ml epidermal growth factor (Collaborative Biomedical Products; Becton Dickinson, Bedford, MA), and 1 mg/ml hydrocortisone (Sigma; Ref. 23). Growth medium was supplemented with 1 mM l-glutamine (Life Technologies, Inc.), 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc.). HUVECs were purchased from BioWhittaker/Clonetics (Walkersville, MD) and cultured in EGM-2 medium (Clonetics). Passages four to six were used in experiments.

The following antibody-producing hybridomas were obtained from American Type Culture Collection (Rockville, MD) and used as culture supernatants: anti-CD4 (L3T4; TIB-207); anti-CD8 (Ly-2.2; TIB-210); and anti-MHC class II (TIB-120). λgM-CSF, μgM-CSF, TNF-α, and VEGF165 were made according to R&D Systems (Minneapolis, MN); polyclonal antimouse immunoglobulin was obtained from Sigma.

All anti-NF-κB antibodies used for EMSA (anti-p50, p52, c-Rel, Rel-A, and Rel-B antisera) as well as anti-IκBe antisera were generous gifts from Dr. N. Rice (ABl-Basic Research Program, National Cancer Institute, NIH). Anti-IκBα, -IκBβ, -IκBγ, -VEGFR2 (Fik1), and -IKKα antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); anti-phosphotyrosine (4G10) was from New England Biolabs, and anti-Ser36 IκBα was from Upstate Biotechnology, Inc.

Portions of mutant and wild-type IκBα (amino acids 1–51) were amplified by PCR from pBS(SK)–IκBα containing full-length wild-type or mutant IκBα. In mutant IκBα, two serines in positions 32 and 36 were substituted by two alanines. PCR products were cloned to pGEX vector, and the constructs were confirmed by sequencing in both directions. GST-IκBα fusion proteins were made according to standard manufacturer’s protocol (Amersham).

Cell Separation. Human CD34+ cells were isolated from umbilical cord blood or bone marrow from the discarded filter sets used for clinical marrow collections by a positive selection technique with CD34 immunomagnetic beads (Dynal, Oslo, Norway) according to the manufacturer’s protocol. The purity of CD34+ cells was >90% as estimated by flow cytometry with an anti-CD34+ antibody (Becton Dickinson). Cells were cultured overnight in complete culture medium (RPMI-1640; Life Technologies; with 100 IU/ml penicillin, 0.1 mg/ml streptomycin, 10 mM HEPES, 5% glycerol, 0.2 mM EDTA, 1 mM magnesium chloride, 20 mM sodium orthovanadate, 50 mM sodium fluoride, 2 mM phenylmethylsulfonyl fluoride, and protease inhibitor tablets (Boehringer Mannheim)) according to Devalaraja et al. (26). The cell lysates (100–150 μg) were cleared and immunoprecipitated by adding anti-IKKB antibodies (Santa Cruz Biotechnology) and protein A/G-agarose (Pierce), with overnight incubation at 4°C. In vitro kinase assay was performed by incubating immunoprecipitates with 4 μg of GST-IκBα (amino acids 1–51) fusion protein in 20 μl of kinase buffer containing 20 mM Tris-HCl (pH 7.6), 10 mM MgCl2, 0.5 mM DTT, 100 μM ATP, and 5 μCi of [γ-32P]ATP for 30 min at 30°C (27). Samples were separated by 10% SDS-PAGE, immunoblotted onto polyvinylidene difluoride membrane (Millipore), and analyzed by autoradiography. Membrane was also probed with anti-IKKα antibodies to check for the equality of loading.

RESULTS

VEGF Effects on NF-κB Activation in Different Types of Cells. Previously, we have reported VEGF binding to one of its specific receptors, VEGFR1, on the surface of hHPCs. This resulted in the inhibition of TNF-α-dependent NF-κB activation in these cells (10). We asked whether VEGF effects on NF-κB were restricted to HPCs or could also be observed in other types of cells expressing the VEGFR1. In this study, we investigated the effect of VEGF on three distinct types of cells: hHPCs, mHPCs, HMEC-1 cells, and RMCs. All of these cell types express mRNA for both TNF-α receptor and VEGFR1 (data not shown). TNF-α induced strong NF-κB activation in all tested types of cells (Fig. 1, Lanes 2 and 3 versus Lane 1). VEGF alone also activated NF-κB in both HPCs and HPCs and RMCs (Fig. 1, Lanes 4 and 5 versus Lane 1); however, this effect was weaker than the effect of TNF-α. No VEGF-inducible NF-κB activation was detected in HMEC-1 cells. In agreement with our data reported previously, VEGF prevented TNF-α-dependent NF-κB activation in human HPCs. The same effect was observed in mHPCs and RMCs (Fig. 1, Lane 6 versus Lane 5). This effect was observed when VEGF was added at the same time with TNF-α or within 10 min before TNF-α. Addition of VEGF 10 min after TNF-α did not affect NF-κB activation (data not shown). No effect of VEGF on TNF-α-inducible NF-κB activation was observed in HMEC-1 cells. These data indicate that the effects of VEGF on NF-κB are not restricted to HPCs.

Effects of VEGF and TNF-α on Nuclear Translocation and DNA Binding of NF-κB Subunits. We asked whether VEGF and TNF-α induced translocation of the same family of the NF-κB family in HPCs. Using supershift EMSA, we found striking differences in the translocation and DNA binding of two members of the NF-κB family, p65 (RelA) and RelB, in response to treatment with VEGF or TNF-α. Significant levels of NF-κB-containing complexes were detected in hHPCs after treatment with TNF-α but not with VEGF (Fig. 2, compare Lanes 2 and 5 in p65 supershift). By contrast, treatment of hHPCs with VEGF resulted in a significant increase in

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nuclear localization could be directly involved in the inhibition of TNF-α-mediated NF-κB activation by VEGF, perhaps by blocking the translocation and DNA binding of complexes containing RelB (Fig. 2, Lanes 2 and 5 in RelB supershift). No differences in the level of p50, p52, or cRel were detected (data not shown). In endothelial cells, TNF-α also induced translocation of predominantly p65-containing complexes, whereas no effect of VEGF was observed (data not shown). Pretreatment of hHPCs with VEGF reduced nuclear translocation and DNA binding of complexes containing p65 (Fig. 2, compare Lanes 2 and 3 in the p65 supershift).

To confirm these differences, nuclear translocation of p65 and RelB subunits of NF-κB was visualized using immunofluorescent confocal microscopy. As shown in Fig. 3, treatment of hHPCs with TNF-α resulted in the accumulation of p65 but not RelB proteins in the nuclei of hHPCs. However, the treatment of these cells with VEGF resulted in nuclear translocation of RelB but not p65 (Fig. 3). To quantitatively analyze these differences, the total number of cells with nuclear localization of the proteins was evaluated in two independently performed experiments using double-blinded observers. Less than 15% of untreated cells demonstrated nuclear localization of p65 or RelB. After treatment of hHPCs with TNF-α, nuclear localization of p65 was found in 95% of cells, whereas nuclear localization of RelB was found only in 35% of cells. Seventy percent of cells treated with VEGF demonstrated nuclear localization of RelB, whereas only 35% of cells had nuclear localization of p65. These data confirm the results obtained by EMSA. Taken together, these data suggest that RelB

Fig. 1. VEGF effects on NF-κB are dependent on cell type. Nuclear extracts were obtained from the cell types indicated. EMSA was performed as described in “Materials and Methods.” The palindromic κB probe was used in experiments with hHPCs and HMEC-1 cells, and the murine intronic κB probe was used in experiments with HPCs and RMCs. Human recombinant TNF-α and human recombinant VEGF were used in experiments with hHPCs and HMEC-1; murine recombinant TNF-α and VEGF were used in experiments with mHPECs and RMCs. Lane C, competition with 100-fold excess of cold probe; Lane 1, control untreated cells; Lane 2, cells treated with 10 ng/ml TNF-α for 10 min; Lane 3, cells treated with 10 ng/ml TNF-α for 20 min; Lane 4, treatment with 100 ng/ml VEGF for 10 min; Lane 5, treatment with 100 ng/ml VEGF for 20 min; Lane 6, 20 min of combined treatment with 100 ng/ml VEGF and 10 ng/ml TNF-α added simultaneously; Lane 7, 20 min of combined treatment with 100 ng/ml VEGF and 10 ng/ml TNF-α (VEGF was added 10 min before TNF-α). At least four experiments with similar results were performed for each cell type.

Fig. 2. VEGF and TNF-α induce the nuclear translocation of distinct NF-κB subunits in hHPCs. Nuclear extracts were obtained from hHPCs, and supershift EMSA was performed as described in “Materials and Methods.” p65 and RelB depict the type of antibody used in supershift. For each antibody: Lane 1, control untreated cells; Lane 2, cells treated with 10 ng/ml TNF-α for 20 min; Lane 3, 100 ng/ml VEGF was added 10 min before TNF-α; Lane 4, 100 ng/ml VEGF was added 10 min after TNF-α; Lane 5, cells were treated with 100 ng/ml VEGF alone for 20 min. Three independent experiments with similar results were performed.
VEGF Inhibits TNF-α-dependent Activation of IKK. The activation of NF-κB by TNF-α involves successive activation of NIK and the IKK complex (29, 30). Serine phosphorylation of the IKKβ subunit by NIK induces its activity and allows it to phosphorylate IκB proteins. We studied the effect of VEGF on IKK activity by in vitro kinase assays using GST-IκBα fusion protein as a substrate. In both hHPCs and mHPCs, VEGF remarkably inhibited TNF-α-induced activation of IKK but did not show any effect when it was added alone (Fig. 8).

VEGFR2 Tyrosine Kinase Inhibition Does Not Block the Inhibitory Effect of VEGF on IKK Activation. We have shown the presence of VEGFR1 mRNA transcripts in human and mouse progenitor cells and specific binding of VEGF to the surface of HPCs (10, 11). Others have detected VEGFR2 mRNA on hematopoietic cells (31). Recently, using antibodies to the VEGFR extracellular domains, distinct populations of HPCs expressing each of the receptors were identified (32–34). To determine whether the effect of VEGF on IKK activation was mediated via the tyrosine kinase activity of VEGFR, we used SU5416, a potent inhibitor of the tyrosine kinase of VEGFR (35, 36). SU5416 completely blocked tyrosine autophosphorylation of VEGFR2 in HUVECs stimulated by VEGF but did not abolish the inhibitory effects of VEGF on IKK activation by TNF-α (Fig. 9).

DISCUSSION

VEGF plays an essential role in both blood vessel formation and hematopoiesis during normal embryonic development. In adult animals, it is induced by local hypoxia and may play a role in wound repair and tissue remodeling. However, increased production of VEGF has been found in many pathological conditions (4–7). An especially important role is attributed to VEGF in cancer. VEGF is
produced by the majority of tumors and plays a critical role in the development of tumor neovasculature. Elevated levels of VEGF in the plasma of cancer patients are closely associated with an adverse prognosis (8, 9). Recently, we have demonstrated that VEGF produced by tumors may also have effects on hematopoiesis and in particular result in the inhibition of functional maturation of dendritic cells, the professional antigen-presenting cells (11, 12). We have demonstrated that a close correlation exists between increased levels of VEGF produced by breast tumor cells and the decreased development of functional dendritic cells. It has been reported recently that elevated levels of VEGF in the vicinity of tumors is associated with decreased tumor infiltration by dendritic cells in patients with gastric cancer (37). Chronic infusion of recombinant VEGF into non-tumor-bearing animals results in dramatic changes in the development of other hematopoietic lineages as well as dendritic cells (12). Thus, VEGF could be an important factor in the abnormal regulation of hematopoietic cell differentiation in a variety of pathological conditions. Understanding the mechanism by which VEGF affects the HPCs could give insights into both normal and pathological hematopoiesis. VEGF is thought to bind to HPCs via the specific receptors VEGFR1 and VEGFR2. This results in inhibition of TNF-α-inducible activation of transcription factor NF-κB (10). Previous data suggested that VEGF might exert its effect via inhibition of NF-κB activation, which is essential for dendritic cell differentiation. In this study, we have tried to elucidate the mechanism of VEGF effects on NF-κB.

We first asked whether the observed effects of VEGF on NF-κB activation were restricted to HPCs or whether they could be observed in other types of cells known to express the receptors, particularly endothelial cells, commonly assumed to be the primary targets for VEGF action. Endothelial cells express all major specific VEGFRs and actively proliferate in response to VEGF. VEGF does not induce appreciable proliferation of other cell types, including HPCs (1, 2, 11, 38, 39). RMCs were also used in these studies. These cells are derived from smooth muscle cell precursors and are a component of the renal glomerulus. We compared the effects of VEGF on HPCs (mouse and human), endothelial cells, and RMCs. All of these cells express both TNF-α and VEGFR1 receptors (41). Not surprisingly, TNF-α induced NF-κB activation in all of these types of cells. VEGF alone also showed some activation of NF-κB DNA binding in HPCs but not in endothelial cells. Using mHPCs and hHPCs, we confirmed our previous observation that VEGF prevented TNF-α-induced NF-κB activation in human HPCs (10). The same effect was observed in RMCs but not in endothelial cells. Thus, the effects of VEGF on NF-κB signaling are dependent on the cellular context.

In an attempt to investigate the mechanism of VEGF effects on HPCs, we compared the profile of NF-κB subunit activation induced by VEGF or TNF-α using a supershift EMSA assay. TNF-α induced nuclear translocation and DNA binding of the complexes containing VEGF-effects on NF-κB activation.
Fig. 9. The VEGFR tyrosine kinase inhibitor SU5416 prevents phosphorylation of VEGFR2 but does not abolish the inhibitory effect of VEGF on IKK activation. Cells were cultured with or without 4 μM VEGF tyrosine kinase inhibitor SU5416 in serum-free medium for 1 h. mHPCs then were treated with 10 ng/ml TNF-α (T), 100 ng/ml VEGF (V), or both together (T + V) for 15 min and lysed. In vitro IKK assay was performed as described in “Materials and Methods.” HUVECs were treated with 100 ng/ml VEGF for 5 min and lysed, and Western blots were performed as described in “Materials and Methods.” Membranes were probed with anti-pTyr antibodies or anti-VEGFR2. C, control untreated cells. Two experiments with similar results were performed for each cell type.

VEGF EFFECTS ON NF-κB ACTIVATION

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+SU5416

predominantly p65. Preferential induction of p65 by TNF-α has been described previously in many reports (42–44). NF-κB complexes induced by VEGF alone also bound to DNA but predominantly contained RelB and not p65, a finding that was confirmed in intact cells by direct nuclear translocation studies.

Our initial hypothesis was that these RelB complexes could be directly involved in the inhibition of p65-containing NF-κB complexes after combined stimulation with TNF-α and VEGF. It was possible that transcriptionally inactive complexes containing RelB could compete for promoter sites normally occupied by p65-containing complexes. Alternatively, RelB release and translocation could be a “bystander phenomenon” associated with failure of TNF-α to induce p65-containing NF-κB complexes in the presence of VEGF. A direct effect of RelB on dendritic cell differentiation was suggested by previous work showing that RelB knockout mice lacked myeloid dendritic cells. However, in this case the lack of RelB was associated with defective dendritic cell differentiation rather than the inappropriate induction of RelB-containing complexes as observed in this study. To determine whether RelB is directly involved in reduction of NF-κB activation by VEGF, we used HPCs obtained from RelB knockout mice. Our experiments demonstrated that VEGF was able to inhibit TNF-α-inducible NF-κB activation in RelB−/− mice similar to the effect observed in RelB+/+ and RelB+/− mice. These data thus argue against a direct inhibitory role for RelB on NF-κB stimulation in this system and imply the existence of other inhibitory mechanisms. Not formally ruled out, however, is the possibility that the lack of RelB has resulted in a loss of a crucial subset of early myeloid committed HPCs in which RelB plays an important role.

What then is the mechanism by which VEGF inhibits the induction of p65-containing complexes? Here we have shown that the VEGF effect is mediated via inhibition of IKK activation in response to TNF-α. Decreased IKK activity prevents efficient serine phosphorylation of IκB and its ubiquitin proteasome-dependent degradation. The large M, ~700,000 IKK complex that phosphorylates IκB proteins in response to proinflammatory signals consists of two catalytic subunits, IKKα and IKKβ, and a regulatory subunit IKKγ (45, 46). Upstream kinases, NIK and mitogen-activated protein kinase kinase kinase 1, are able to phosphorylate both IKK catalytic subunits (27, 47); however, only IKKβ phosphorylation contributes to IKK activation by TNF-α (29). The proposed mechanism of IKK function suggests that the IKKβ subunit contains sites for both positive and negative regulation. IKKβ is rapidly activated through phosphorylation of its “activation loop” (T loop) by upstream kinases, and this is followed by progressive autophosphorylation at a COOH-terminal serine cluster, which in turn inhibits catalytic activity (29). VEGF may exert its inhibitory action by blocking the initial phosphorylation of serines in the T loop of IKKβ or by causing its rapid dephosphorylation. This mechanism would imply participation of VEGF-activated phosphatase(s). Phosphatases SHP1 and SHP2 (48) and HCPTPA (49) have been proposed to be an integral part of the VEGF receptor signaling pathway, playing a role in the down-regulation of the VEGF signal. It is possible that VEGF-related phosphatases may interfere with other signaling pathways, in particular with that of NF-κB. The potential involvement of phosphatases in the regulation of NF-κB signaling was demonstrated recently by association of the c-Rel subunit of NF-κB with serine/threonine protein phosphatase 4 (also known as PPIX; Ref. 50). Alternatively, VEGF may enhance subsequent phosphorylation of the COOH-terminal serine cluster, leading to IKK deactivation. Upstream NIK kinase activity may also be a target for VEGF effect. With the current data, it is not possible to discriminate between these possibilities, and these hypotheses are under investigation.

It is interesting to note that although VEGF induces DNA binding of RelB containing dimers, we were unable to demonstrate its effect on IκB proteins, especially IκBα and IκBε, which are readily degraded upon TNF-α stimulation. One possible explanation is that IκBα has high affinity for p65-containing complexes (14) but much less affinity for RelB-containing complexes, which are also less susceptible to inhibition by IκBα (51, 52). VEGF could also induce tyrosine phosphorylation of IκBα or IκBε and dissociation of NF-κB complexes without IκB degradation, the mechanism described recently by Imbert et al. (15). However, we could not detect tyrosine phosphorylation of IκBα induced by VEGF. Not excluded is the possibility that in HPCs, RelB is predominantly associated with another, as yet unknown, member of the IκB family, resulting in RelB release upon stimulation with VEGF but not TNF-α.

VEGFR2 exercises its important functions through autophosphorylation and activation of its receptor tyrosine kinase domain upon ligand binding (53, 54). Less is known about the VEGFR1 signal transduction pathway. However, data suggest that the kinase domain of this receptor plays a much less important role, and its function is realized rather through other mechanisms. Thus, although VEGFR1 knockout mice die in early embryonic development because of disorganization of blood vessels, VEGFR1 tyrosine kinase-deficient homozygous mice survive without developing an observed phenotype (53). To study the role of receptor tyrosine kinase activity in VEGF-induced effects on HPCs, we used VEGFR kinase inhibitor SU5416. This inhibitor potently prevents phosphorylation of both VEGFR1 and VEGFR2, activation of downstream signaling from VEGFR2, proliferation of endothelial cells, and angiogenesis in vivo (35, 36). In our control experiments, SU5416 completely blocked VEGFR2 autophosphorylation in HUVECs, as expected. However, at the same concentration, it was ineffective in reversing the VEGF inhibitory effect on IKK activity. This indicates that activity of the VEGFR tyrosine kinases is not involved in the observed effects on NF-κB. It is most likely mediated by alternative mechanisms linked to VEGF in agreement with our previous data (10) or an as yet undescribed receptor.
We have therefore identified two unique effects of VEGF on NF-κB activation: nuclear translation and DNA binding of complexes containing predominantly RelB, and receptor tyrosine kinase-independent inhibition of TNF-α-dependent activation of IKK, leading to a decrease in IκBα serine phosphorylation and degradation of IκBα and IκBε. These studies may have identified a new mechanism for the regulation of NF-κB and for VEGF effects on NF-κB in hematopoietic progenitor cells.

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