Vascular endothelial growth factor promoted endothelial progenitor cell mobilization into the peripheral blood of a patient with POEMS syndrome

We assessed the percentage of endothelial progenitor cells (EPCs) in the peripheral blood of a patient with POEMS with elevated VEGF plasma levels. High VEGF plasma levels were associated with increased EPC concentration and treatment with an anti-VEGF antibody induced a consensual decrease of both parameters. *In vitro* cultures of the patient BM cells suggested that the stromal compartment could be responsible for VEGF overproduction.

Haematologica 2007; 92:1291-1292. DOI: 10.3324/haematol.11455

There is increasing evidence that high levels of vascular endothelial growth factor (VEGF) in the peripheral blood (PB) of patients with POEMS syndrome could account for organomegaly, skin lesions, and edema.[1-3] Since nothing is known on the effect exerted by elevated concentrations of VEGF on circulating endothelial progenitor cells (EPCs) in this disease, we studied this cell population in a POEMS patient who presented with high VEGF levels, and investigated the source of the cytokine synthesis.

Serum VEGF was assessed by ELISA (R&D Systems, Minneapolis, MN, USA) and the percentage of circulating EPCs by cytofluorimetric analysis, using antibodies directed against CD34 (Becton Dickinson, Pharmingen, San Jose, CA), CD133 (Miltenyi Biotech, Bergisch Gladbach, Germany), and VEGFR-2 (Sigma Chemicals, St. Louis, MO) antigens,[4] before and after treatment with Bevacizumab (Avastin, Roche, Basel, Switzerland), a humanized anti-VEGF monoclonal antibody that has been occasionally employed for the treatment of POEMS syndrome.[5,6] As reported in Table 1, the VEGF level and the percentage of circulating $CD34^+$ $CD133^+$ $VEGFR2^+$ EPCs before treatment were higher than in normal subjects. Bevacizumab infusion induced a quick reduction of serum VEGF concentration and a specific decrease of circulating EPCs, [$CD34^+$ hematopoietic progenitor cells (HPCs), mature circulating $CD146^+$ endothelial cells (ECs) and mature $Mac\text{-}1^+$ myeloid cells were unchanged] (Table 1). In order to define the source of VEGF overproduction in this patient, we assessed the levels of VEGF and IL-6 (another cytokine known to be increased in POEMS syndrome)[1] in the supernatant of *in vitro* cultured BM stromal cells obtained just before Bevacizumab infusion. BM stromal layers were established in the presence of Myelocult (StemCells Technologies Inc, Vancouver, BC, Canada),[7] and VEGF and IL-6 levels assessed by ELISA (R&D System) in 24, 48, 72, 96 and 1 week culture supernatants. Whereas IL-6 levels did not change within 1 week of culture (ranging between 676 and 731 pg/mL), we found a progressive, remarkable increase of VEGF levels from 24 hours of culture to 1 week (from 0.75 to 854 ng/mL, respectively; Table 2). VEGF levels in similar experiments performed with BM stromal layers from a patient with MGUS and 2 healthy subjects, used as a negative controls, were low (range: 0.15-0.34 ng/mL) and remained unchanged within 1 week (Table 2). Finally, supernatant of cultures of immunomagnetically selected $CD138^+$ cells (Miltenyi Biotec) from our patient and the patient with MGUS did not show any assayable difference of VEGF levels.

Table 1. Serum VEGF concentrations and circulating cell subsets before and after Bevacizumab infusion.

	SerumVEGF (pg/mL)*	WBC ($x10^3$/mcL)	EPCs†	HPCs‡	$CD146^+$ cells§	$Mac\text{-}1^+$ cells^
Pre-treatment	1756	3.94	0.18	0.02	0.4	38
5 days after 1st infusion	159	4.76	0.000	0.02	0.00	33
3 days after 2nd infusion	151	5.2	0.003	0.01	0.8	36
1 week after 2nd infusion	98	6.2	0.000	0.02	0.00	26

*Range of normal values: <15.6-115 pg/mL. †expressed as percentage of total circulating mononucleated cells; normal range 0-0.004%. ‡Expressed as percentage of $CD34^+$ cells according to ISHAGE guidelines. §Mature endothelial cells, expressed as percentage of total circulating mononucleated cells; normal range: 0-1%. ^Expressed as percentage of total circulating mononucleated cells; normal range: 15-35%.

Table 2. VEGF and IL-6 levels in the supernatant of BM stromal cell cultures at different time points.

	POEMS patient		MGUS patient		Healthy subjects (n=2)*	
Time of culture	VEGF (ng/mL)	IL-6 (pg/mL)	VEGF (ng/mL)	IL-6 (pg/mL)	VEGF (ng/mL)	IL-6 (pg/mL)
24 hours	0.75	676.92	<0.15	46.87	< 0.15	ND°
48 hours	1.98	ND	<0.15	ND	ND	ND
72 hours	2.17	688.45	0.27	166.11	< 0.15	ND
96 hours	166.20	672.41	0.33	ND	0.21	ND
1 week	854.50	731.41	0.26	198.86	0.19	ND

*Values shown are the average of two individual experiments performed with BM of two healthy volunteers. °ND: not done:

There is growing evidence that high levels of serum VEGF play a role in the pathogenesis of some clinical manifestations of POEMS. For instance, VEGF may increase micro-vascular hyperpermeability, leading to edema, increased endoneural pressure and thereby to neural damage.[1,2] We demonstrate for the first time that high levels of serum VEGF are associated with high percentages of circulating EPCs in this disease and that treatment with Bevacizumab induces a rapid decrease of both these parameters (with HPC, mature ECs and mature myeloid cells unchanged before and after treatment). This observation indicates a role for VEGF in mobilizing EPCs into the PB in this disease, in keeping with the notion that VEGF can mobilize BM-derived EPCs in an animal model,[8] and in human subjects.[9] In addition, it suggests a potential involvement of circulating EPCs in the neoangiogenetic processes, which has been described to be increased in POEMS patients and likely involved in the pathogenesis of POEMS polyneuropathy.[10] Whether or not exposure to chronically elevated levels of VEGF can affect not only the number of circulating EPCs but also their functional activity

remains to be investigated. Finally, the observation that BM stromal cells, which presumably are not part of the malignant clone of the disease, produced high amounts of VEGF *in vitro* suggests that an aberrant response of BM microenvironment to the abnormal clone could be responsible for VEGF overproduction in POEMS patients. Experiments on a larger number of patients will clarify the mechanism(s) by which the malignant clone and BM microenvironment interact in POEMS patients.

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