International Immunology, Vol. 21, No. 9, pp. 1057–1063
doi:10.1093/intimm/dxp070

Anti-vascular endothelial growth factor (VEGF) specific activity of intravenous immunoglobulin (IVIg)

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Keywords: autoimmunity, cancer, innate immunity, IVIg, VEGF

Abstract

Intravenous immunoglobulins (IVIg) preparations can be beneficial therapeutic agents for the treatment of tumor metastases as has been shown in both human and animal studies. Operating mechanisms have not yet been completely elucidated. Some of the mechanisms proposed entail the stimulation of the production of IL-12, a cytokine that exhibits anti-angiogenic activities, as well as inhibition of endothelial cells proliferation and vascular endothelial growth factor (VEGF) secretion. The aim of the present study was to investigate whether in an IVIg preparation there are natural antibodies directed against VEGF with the potential to affect angiogenesis. Using both sandwich and direct ELISA assays, IVIg was found to specifically recognize and bind VEGF in a dose-dependent manner. The binding specificity was confirmed by inhibition of IVIg binding to VEGF by VEGF as an inhibitor, as shown by ELISA and immunoblot. A mouse hind limb ischemia model was employed to evaluate the *in vivo* IVIg-induced inhibition of angiogenesis. IVIg was found to exhibit inhibitory effect on VEGF-mediated blood perfusion in the ischemic limb. The present study shows a presence of anti-VEGF fraction in IVIg preparation.

Introduction

Angiogenesis, a multistep process that results in the formation of new blood vessels from pre-existing vasculature, is essential for both tumor growth and metastasis (1–3). Tumor-associated angiogenesis is mainly regulated by the vascular endothelial growth factor (VEGF) whose over-expression has been demonstrated in many human tumors (4–6). Therapeutic targeting of VEGF and vascular endothelial growth factor receptors (VEGFRs) inhibits tumor growth and invasiveness as shown in both pre-clinical and clinical studies (7–10). A number of new therapies have been developed and employed in treating patients with cancer to counteract the pathogenic effects of VEGF. These treatments include toxic drugs such as VEGFR tyrosine kinase receptor inhibitors (11), soluble receptors, which prevent VEGF from binding to its normal receptors (12), and mAbs against VEGF or VEGFR (13, 14). Notably, all of them have been used in clinical trials and demonstrated in patients the ability to directly target the blood supply of tumors, leaving healthy tissues unharmed.

The discovery and development of bevacizumab, the first angiogenesis inhibitor to be approved by the US FDA as a first-line therapy for colorectal cancer, followed recently by the VEGFR tyrosine kinase inhibitors sorafenib and sunitinib, address the potential of a novel approach to cancer treatment (15–17). Although mAbs to VEGF and VEGFR, such as bevacizumab and trastuzumab, have become an important component of the cancer treatment, such therapy is still expensive and cumbersome. These limitations have prompted scientists to investigate alternative strategies.

Intravenous immunoglobulins (IVIg) are therapeutic preparations of normal human IgG obtained from plasma of several thousand healthy blood donors. They are initially used as replacement therapy for a wide range of primary and secondary immunodeficiency states and thereafter for treatment of different autoimmune as well as systemic inflammatory diseases (18–21). Furthermore, in several experimental and clinical studies, it was shown that IVIg exhibited inhibitory effect on tumor spread and that IVIg gamma globulins may be a supportive therapy for the treatment of metastatic cancer and prolongation of disease-specific survival (22–30). Recently, using both *in vitro* and *in vivo* methods, we

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Received 29 March 2009, *accepted* 29 June 2009

Transmitting editor: I. Pecht

Advance Access publication 22 July 2009

reported that IVIg inhibited both CT26 colon carcinoma cell proliferation and invasion through an extracellular matrix and that systemic administration of IVIg into tumor-bearing mice was significant effective in attenuating tumor growth and decreasing the number and size of lung metastases (31). We also showed that intra-corneal injection of IVIg to CT26 cell-implanted rabbit corneas led to shrinking and complete disappearances of tumor mass in 10 days (31). Several mechanisms of action of IVIg have been proposed [reviewed in ref. (32)] including: (i) down-regulation of metalloproteinase-9 (33), the extracellular matrix-degrading enzyme important for tumor growth and invasiveness; (ii) activation of NK cells to produce antibody-dependent cellular cytotoxicity activity against tumor cells (28); (iii) stimulation of the production of IL-12 (24), a cytokine that exhibits anti-angiogenic activities and (iv) finally, inhibition of endothelial cells (ECs) proliferation, VEGF mRNA expression and protein secretion, as well as vascular cell adhesion molecule-1 production (34). The ability of IVIg to down-regulate the cytokine production by ECs and activation of vascular endothelium indicates that possible mechanism of action of IVIg antibodies on metastatic colon carcinoma may lie in their interference with VEGF pathway of tumor angiogenesis. Thus, VEGF and its receptors seem to be a suitable target for therapeutic intervention with IVIg.

The aim of the present study was to examine if in an IVIg preparation there are antibodies directed specifically to VEGF with the potential to inhibit tumor vascularization and to reduce angiogenesis. Demonstrating such anti-VEGF activity in IVIg would be helpful in further understanding the mechanisms by which IVIg affects tumor metastatic spread, as well for providing some additional supportive treatment strategies to fight cancer.

Methods

Immunoglobulins

IVIg used in the study was kindly provided by Omrix Biopharmaceuticals Inc., Nes-Ziona, Israel. Anti-VEGF mAbs—mouse-anti-VEGF mAb (ProSpec-Tany Technogene, Rehovot, Israel) and bevacizumab, a humanized anti-VEGF mAb (Avastin; Roche Pharmaceuticals). Normal human IgG was affinity purified from serum of healthy donor on a protein G column (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the manufacturer's instructions.

Biotinylation of antibodies

IVIg and anti-VEGF mAb in bicarbonate buffer, pH 8.5, were biotinylated with sulfo-NHS-biotin (Sigma), for 4 h and dialyzed against PBS.

VEGF

Recombinant human vascular endothelial growth factor (rhVEGF) (ProSpec, Rehovot, Isreal).

Binding of IVIg to the recombinant VEGF (sandwich ELISA)

Plates were coated with 1 μg ml^{-1} mouse anti-VEGF antibody (DuoSet ELISA, R&D System), washed and blocked before adding rhVEGF. An ELISA was carried out using biotinylated intravenous immunoglobulins (b.IVIg) followed by streptavidin–HRP and appropriated substrate. Data were read at a wavelength of 450 nm.

Binding of IVIg to the solid-phase-immobilized VEGF

rhVEGF (0.5 μg ml^{-1} in PBS) -coated ELISA plates were blocked with 3% BSA. Serial dilutions of b.IVIg, biotinylated anti-vascular endothelial growth factor (b.anti-VEGF) mAb or biotinylated control-IgG were added and probed as described for sandwich ELISA.

Inhibition of IVIg–VEGF binding by VEGF

b.IVIg, at 50% binding VEGF (EC_{50}), was incubated in the presence of different concentrations of VEGF, at 4°C 16 h. The reaction mixture was subjected to the VEGF, in the sandwich ELISA, as described above.

Blocking of IVIg binding to VEGF in a direct ELISA

For the assessment of the IVIg capability to prevent the binding of anti-VEGF mAb to VEGF, VEGF-coated plates were incubated with different concentrations of IVIg, and thereafter, mouse or human b.anti-VEGF mAbs (at EC_{50}) were added. The percentage of inhibition of binding was calculated.

Binding of IVIg to the recombinant VEGF (immunoblot)

rhVEGF samples were loaded onto 12% SDS–PAGE (1 μg per lane) and run under non-reducing conditions. The protein was transferred to nitrocellulose membrane, blocked with 10% skim milk in Tris-buffered saline (TBS). Both b.anti-VEGF mAb (mouse or human) and b.IVIg, diluted in 0.5% skim milk–TBS, were added to the membrane for 2 h. For inhibition assays, either disabling of IVIg-to-VEGF binding by anti-VEGF mAb or conversely, the prevention of anti-VEGF mAb to VEGF reaction by IVIg was evaluated. The binding to VEGF was probed with streptavidin–HRP followed by an appropriate substrate (ECL-Luminol Reagent, Santa Cruz, CA, USA).

Anti-VEGF activity of IVIg in a model of mouse hind limb ischemia

The mouse ischemic hind limb model (35–37) was used to evaluate the inhibitory effect of IVIg on VEGF-mediated reperfusion.

Mice. Inbred 12- to 14-weeks-old C57BL/6 female mice were purchased from Harlan labs (Rehovot, Israel). All experimental procedures were reviewed and approved by the Animal Care and Ethical Committee, Tel Aviv University.

Hind limb ischemia. To create an unilateral hind limb ischemia, the surgical procedure was performed as described (35). Briefly, an incision in the skin overlying the middle portion of the mouse left hind limb was performed and the femoral artery was exposed and ligated. Next day, a group of mice received a single intramuscular injection of VEGF (500 ng per mouse) in order to attain the blood perfusion in the ischemic limb. Control mice were injected with PBS. Forty-eight hours later, some of the VEGF-injected mice (20 of 28) were treated with 1 g kg^{-1} of IVIg, intravenously.

Hind limb blood perfusion. Laser Doppler Perfusion Imaging (Perimed, Sweden) was used to record serial blood flow measurements beginning at the time of surgery and over the course of 3 weeks post-operatively. Analyses were performed by determining the median perfusion values for each foot and calculating the ischemic (left) versus normal (right) limb blood perfusion ratio (relative units). Results were presented as the mean ± SEM.

Results

Determination of IVIg anti-VEGF activity

Anti-VEGF antibody activity in an IVIg preparation was determined using a quantitative sandwich enzyme immunoassay and behaved in a dose-dependent manner (Fig. 1A). Direct binding of IVIg-to-VEGF-coated ELISA plates showed a dose response as well (Fig. 2A). The calculated 50% effective concentration (EC_{50}) value for IVIg was ~12.5 mg ml^{-1}. The data were confirmed by western immunoblot. IVIg preparation revealed an intense band, migrating at 38 kDa, which corresponds to the molecular mass of one of VEGF isoforms ($VEGF_{121}$). Strong, positive reaction was noticed using 2 mg ml^{-1} of IVIg, whereas a lower concentration of 2 μg ml^{-1} did not yield a clear pattern of binding (Fig. 1B).

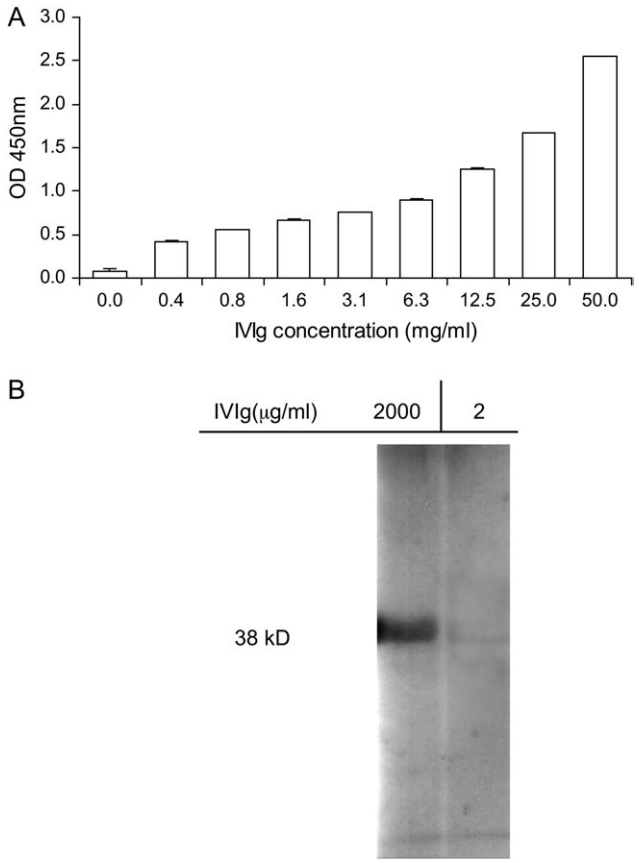

Fig. 1. Binding of IVIg to rhVEGF. (A) Sandwich ELISA, using mouse-anti-human-VEGF-coated plates, VEGF and probed with biotinylated IVIg. (B) b.IVIg binding to VEGF by immunoblot. b.IVIg 2 mg ml^{-1} (left line) and 2 μg ml^{-1} (right line).

IVIg–VEGF binding in a solid-phase ELISA

The results presented in Fig. 2(A) of an ELISA assay with VEGF-pre-coated plates revealed a dose-dependent direct binding of IVIg-to-VEGF, thus confirming results obtained by sandwich ELISA (see above). The minimum detection limit estimated by the serial dilutions was ~10 μg ml^{-1} and EC_{50} value was found to be 2.35 mg ml^{-1}. For the assessment of specificity and sensitivity of IVIg–VEGF binding, anti-VEGF mAbs were employed as positive controls. As seen in Fig. 2(B), anti-VEGF mAb bound to the VEGF-coated plates in a dose-dependent fashion and its minimal detection limit estimated by serial dilutions was ~10 ng ml^{-1}. Its determined that EC_{50} value was 5 μg ml^{-1} for human and 3 μg ml^{-1} for mouse antibody and this concentration was used in the inhibition assay of IVIg to VEGF binding. Additionally, the relative capabilities of IVIg and those of anti-VEGF mAb and single person IgG (used as positive and negative controls, respectively) to bind immobilized VEGF were compared. For that purpose, two different concentrations (10 and 1 μg ml^{-1}) of all three preparations were set against and tested for their binding capabilities (Fig. 3). It was found that anti-VEGF mAb exhibited a significantly greater binding potential than IVIg (Fig. 3A). On the other hand, for all tested concentrations, IVIg showed a higher binding efficacy than the single donor IgG, which otherwise showed a minimal activity (Fig. 3B).

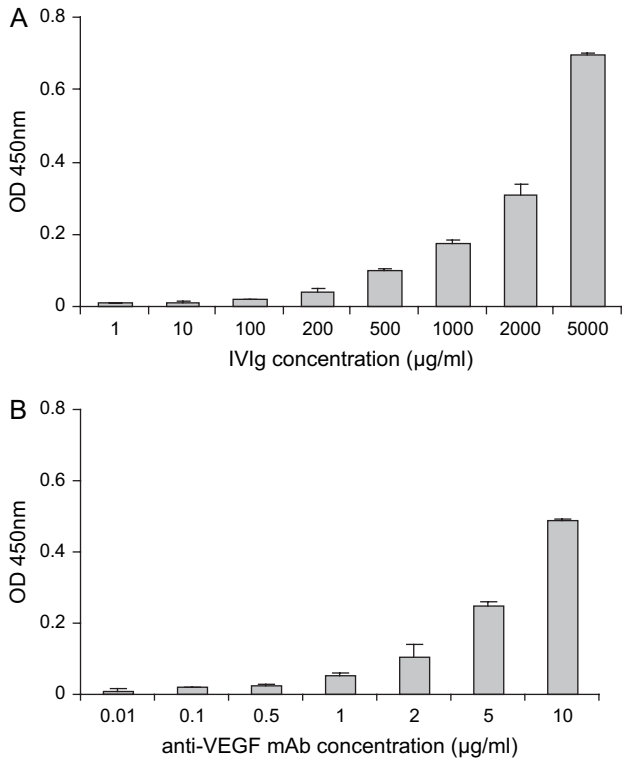

Fig. 2. Anti-VEGF activity in a direct solid-phase-immobilized VEGF ELISA testing. VEGF was coated on an ELISA plate at 0.5 μg ml^{-1}. Serial dilutions of both biotinylated IVIg (A) and anti-VEGF mAb samples (B) binding to rhVEGF-coated plates. Values represents means ± SEMs of three independent experiments.

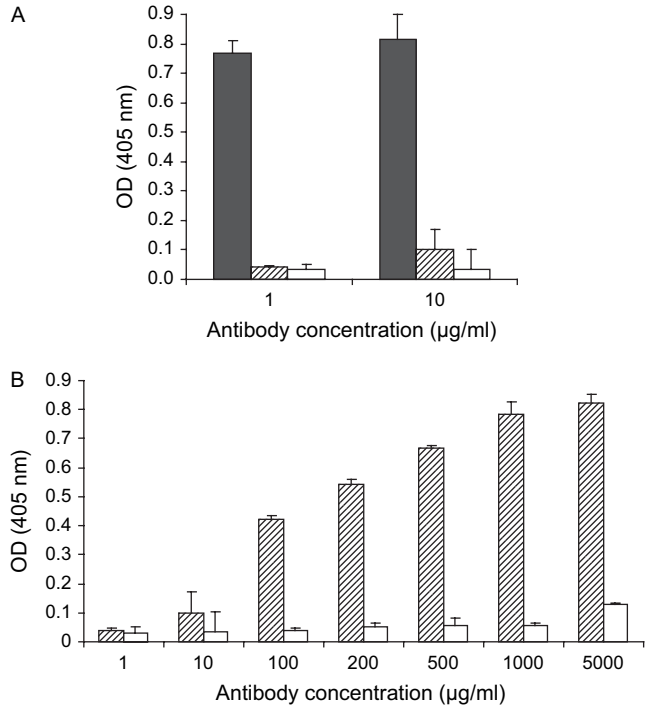

Fig. 3. Anti-VEGF antibody level in an IVIg preparation in comparison with m.anti-VEGF: IVIg (striped columns), anti-VEGF mAb (black columns) and IgG from one individual (white columns). All three preparations were compared with each other in two concentrations (10 μg ml^{-1} and 1 μg ml^{-1}) (A). Dose response of IVIg anti-VEGF activity in comparison with single person IgG (B). Data presented are the mean ± SEM of duplicates.

Specificity and sensitivity of IVIg binding to VEGF

Inhibition of IVIg–VEGF binding by VEGF. Pre-incubation of IVIg with different doses of soluble VEGF, used as an inhibitor, resulted in the reduction of IVIg-to-VEGF interaction in the sandwich ELISA (Table 1). Results indicated that all tested concentrations of a soluble VEGF were able to inhibit interaction between an IVIg and the VEGF captured by an anti-VEGF antibody.

Inhibition of IVIg anti-VEGF reaction to VEGF. To estimate the specificity of IVIg-to-VEGF recognition, either inhibition of IVIg-to-VEGF binding by anti-VEGF mAb or inversely, inhibition of anti-VEGF mAb binding to-VEGF by IVIg was carried out. For this purpose, in one set of experiments, ELISA plate-immobilized VEGF was saturated with mouse-anti-VEGF mAb used as an inhibitor, before adding the b.IVIg, whereas in the other one, IVIg was used as an inhibitor and subjected to the solid face-attached VEGF before adding the b.human-anti-VEGF mAb. The percentage of inhibition was calculated and presented in Fig. 4. As shown, the inhibition rate of IVIg anti-VEGF activity was 17.1, 10.0, 3.9 and 2.4%, when human-anti-VEGF mAb was used as an inhibitor at concentrations of 5, 2, 1 and 0.1 μg ml^{-1}, respectively (Fig. 4A). On the other hand, as depicted in Fig. 4(B), IVIg used as an inhibitor at concentrations of 5, 1, 0.2 and 0.05 mg ml^{-1} exhibited a higher reduction but still not completely blocking activity (56, 42.1, 9.8 and 1.5%, respectively). The same set of blocking of binding to VEGF experiments was performed also by western blot analysis (Fig. 4C). Results obtained from an immunoblot confirmed that there were specific bindings of both IVIg and anti-VEGF mAb to the VEGF (Fig. 4C lines 3 and 4, respectively) and that each of them were able to partially block the binding potential of another one (Fig. 4C lines 1 and 2).

Table 1. Inhibition of IVIg-to-VEGF binding by pre-incubation of IVIg with different concentrations of VEGF in the sandwich ELISA

VEGF concentration	Absorbance 450 nm	% Inhibition
0	1.249	
0.1	1.044	16.41
0.5	1.001	19.86
1	0.835	33.15
2	0.141	88.71
4	0.105	91.59
8	0.059	95.28

Concentration of IVIg was 12.5 mg ml^{-1} (EC_{50} value). Presented results are mean values of three separate experiments

Inhibition of VEGF-induced ischemic hind limb blood perfusion by IVIg

A mouse hind limb ischemia model was used to evaluate the *in vivo* inhibition of angiogenesis induced by IVIg. Ischemic mouse hind limbs were treated with PBS, VEGF alone or VEGF together with IVIg. The average perfusion of each limb was computed and the results were expressed as the ischemic (left) versus control (right) blood perfusion ratio. A statistical analysis demonstrates a significant increase in the blood perfusion ratio in the VEGF-injected group in comparison with the mice injected with PBS, 14 days after the surgery. At the same time, the IVIg administration resulted in 15% of blood perfusion inhibition in VEGF-injected mice (Fig. 5).

Discussion

By benefits of the diverse repertoire of Ig with a wide spectrum of specificities, IVIg preparation represents a promising strategy for the treatment of immunodeficiency states and different autoimmune as well as systemic inflammatory diseases (18–21). Autoimmunity and malignancy co-exist frequently and share several common features, which brought up an idea that IVIg may also help for treatment of cancer [reviewed in ref. (38)]. IVIg treatment has been shown to reduce metastases and tumor recurrence for a broad spectrum of cancers, with virtually no side effects (39). Moreover, it appears to increase the chances for long-term recovery by preventing the return and spread of cancer, as demonstrated in animal models, several case reports and clinical studies (25, 26).

Based mainly on the animal studies, it was suggested that IVIg exert beneficial anti-tumor effect, resulting in metastases suppression via different mechanisms [reviewed in ref. (32)]. These mechanisms include direct antibody-mediated cytotoxicity, activities against tumor and EC adhesion

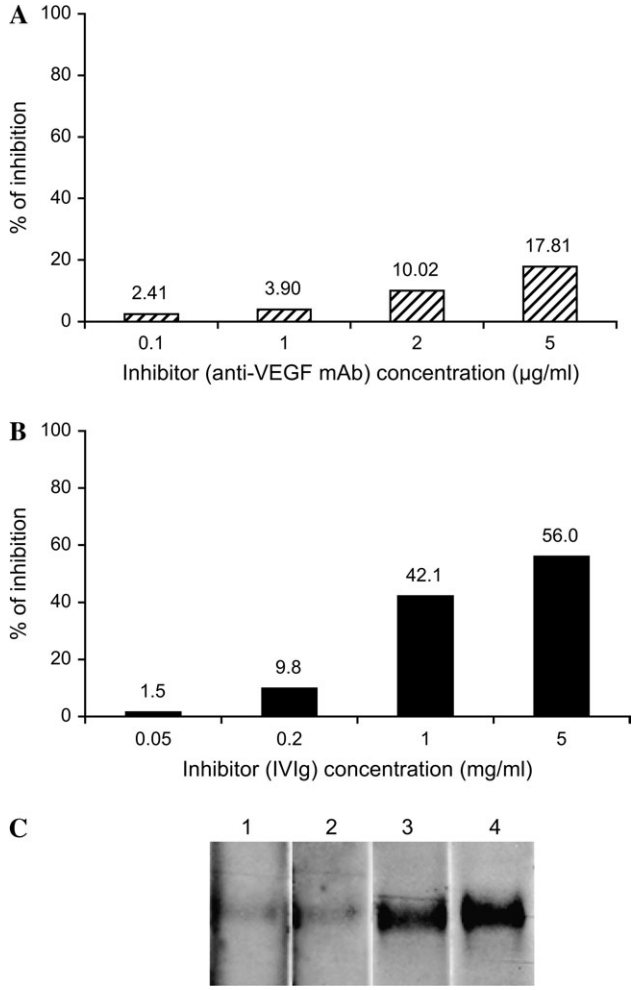

Fig. 4. Blocking the anti-VEGF activity in both direct ELISA and immunoblot assays. (A) An inhibition assay was performed using biotinylated IVIg at EC_{50} (2.35 mg ml^{-1}) to detect immobilized VEGF (500 ng ml^{-1}) in the presence of anti-VEGF mAb (inhibitor) at varying concentrations. Inversely, (B) biotinylated mouse anti-VEGF mAb at EC_{50} (3 μg ml^{-1}) was used to detect VEGF in the presence of different concentrations of IVIg as an inhibitor. Graphs show the percentage of inhibition of binding to the VEGF as the average of three independent experiments. (C) For the same set of experiments performed in an immunoblot, Lane 1: Binding of IVIg (2 mg ml^{-1}) to the VEGF pre-incubated with anti-VEGF mAb (10 μg ml^{-1}). Lane 2: Binding of anti-VEGF mAb (10 μg ml^{-1}) to the VEGF pre-incubated with IVIg (2 mg ml^{-1}). Lane 3: Direct binding of IVIg (2 mg ml^{-1}) to the VEGF. Lane 4. Direct binding of anti-VEGF mAb (10 μg ml^{-1}) to the VEGF. The anti-VEGF mAb used in the western blot presented above was humanized anti-VEGF mAb.

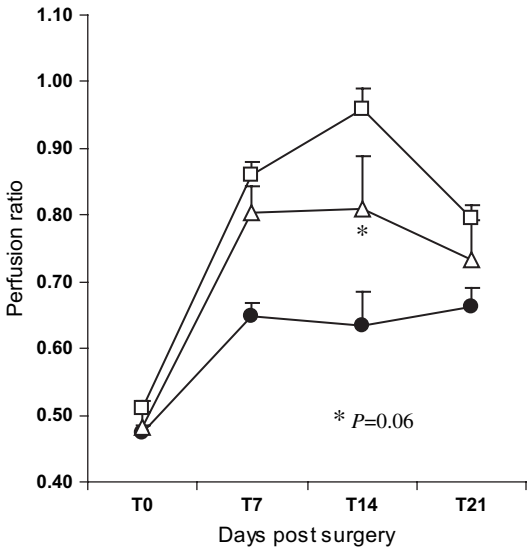

Fig. 5. Inhibitory effect of IVIg on VEGF-induced blood perfusion recovery in a murine model of hind limb ischemia. Quantitative analysis of blood perfusion ratio over the course of 3 weeks post-ischemia/reperfusion induction in the left femoral artery. Ischemic hind limb mice were treated either with VEGF (perfusion) or with PBS (ischemia alone) or with VEGF followed by IVIg injection. VEGF (or PBS, as a control) was administrated 24 h following ischemia, and IVIg 48 h afterward. The blood flow measurements were determined by Laser Doppler perfusion imaging and the ratio (operated versus non-operated leg) was calculated for each animal. Results were presented as the mean ± SEM. The statistical significance of differences among the results obtained was analyzed using the two-tailed, unpaired Student's *t*-test. A difference was considered statistically significant when the *P* value was <0.05. Closed circle: without VEGF (ischemia alone); square: with VEGF (perfusion); triangle: with VEGF + IVIg.

molecules, stimulation of different cytokine secretion, as well as inhibition of secretion of extracellular matrix degrading enzyme that is important for tumor growth and invasiveness. Furthermore, one must remember that metastasis is an angiogenesis-dependent process (1) and that the role of IVIg in attenuation of tumor spread (31) could also be the result of decreasing the blood supply to the tumor. Hence, it was found that IVIg stimulate the production of IL-12, an anti-angiogenic cytokine, from the healthy blood donor's mononuclear cells (24). Moreover, IVIg may generate apoptosis to inhibit vascular EC proliferation and VEGF mRNA expression (34, 40, 41). Despite that, the possible effects of IVIg on tumor blood supply, particularly, on VEGF-induced angiogenesis have not been studied yet. We hypothesized that in an IVIg preparation, there are antibodies directed specifically to the VEGF with the potential to bind to- and neutralize its bioactivity and consequently inhibit tumor vascularization. Results of the present study have indeed demonstrated that gamma globulins from an IVIg preparation contained sub-fractions capable to recognize and bind to the VEGF. These sub-fractions are prevalent in preparations obtained from a large plasma pools but undetectable in preparations from a single individual (Fig. 3). Their specificity for VEGF was proven by VEGF-induced inhibition of IVIg-to-VEGF interaction as well as by competitive inhibition with the commercial anti-VEGF mAb (Table 1 and Fig. 4, respectively).

The regulatory role of VEGF in therapeutic angiogenesis was first suggested by experiments in which supplemental VEGF, administered as recombinant protein or naked DNA, augmented collateral blood flow in several animal models of hind limb ischemia (35, 42). Employing the same kind of mouse model, we tested the possibility that IVIg-anti-VEGF antibodies are functionally active and may inhibit angiogenic properties of VEGF *in vivo*. As shown, under the defined experimental conditions, quantitative analysis of the laser Doppler imaging has disclosed

a significant increase in blood perfusion in the ischemic limb after VEGF injection in comparison with controls receiving infusion of PBS. On the contrary, following IVIg administration, the relative blood flow in an ischemic limb, measured after 14 days of ischemia/VEGF-mediated reperfusion, was decreased by 15%.

These results provide evidence for functional anti-VEGF activity in IVIg capable to inhibit the VEGF-induced angiogenesis. The blood perfusion has not been significantly inhibited ($P = 0.06$). The presence of relatively low concentration of anti-VEGF antibodies in an IVIg preparation may lead to an expected more pronounced effect of affinity fractionated specific IVIg-anti-VEGF. In that case, it would make it possible to inject these purified/specific antibodies in much lower concentration, than the whole IVIg. Subsequently, such antibodies would be expected to be much more effective, as shown for both lupus anti-anti-dsDNA and antiphospholipid syndrome anti-anti-β2GPI antibodies fractionated from the IVIg (43–45). Another probable explanation that the effect of IVIg on the blood flow was not pronounced enough is due to the small animal size. Namely, the vascular growth and remodeling processes in mice can be markedly different from that in humans, where the effect could be much more prominent (46).

The validity of our assumption that IVIg may affect VEGF production and/or expression and to contribute, at least partially, to the inhibition of angiogenesis was strongly supported recently by findings of the effect of IVIg on activated human vascular ECs (34). Authors clearly showed that IVIg was able to inhibit EC proliferation and significantly downregulated the VEGF and adhesion molecule production and mRNA expression. Although in the present study, we did not determine the inhibitory effect of IVIg on the VEGF bioactivity and tumors vascularization, our previous results regarding the effect of IVIg on colon carcinoma might support this hypothesis (31). Namely, we have shown that IVIg treatment of rabbit eye corneas, inseminated with colon carcinoma cells, led to the shrinking of the tumor mass and to the complete disappearances of the rich vascular bed.

In this paper, we provide the first evidence for the presence of biologically active anti-VEGF antibodies in an IVIg preparation. We are aware of the fact that different preparations of IVIg may have diverse biological activities, a point that needs further analyses. Our results may contribute to a possible role of IVIg as natural auto-antibodies and may have a supportive therapy for chemotherapy in cancer, precluding patients with kidney afflictions. In addition, our results of VEGF-specific activity of IVIg can also make important contribution to our understanding of the role of IVIg in the therapy of different autoimmune conditions (47).

Funding

Israeli Cancer Foundation (2009003).

Abbreviations

b.anti-VEGF	biotinylated anti-vascular endothelial growth factor
b.IVIg	biotinylated intravenous immunoglobulins
EC	endothelial cell
IVIg	intravenous immunoglobulins
rhVEGF	recombinant human vascular endothelial growth factor
TBS	Tris-buffered saline
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor

References

1 Folkman, J. 1971. Tumor angiogenesis: therapeutic implications. *N. Engl. J. Med.* 285:1182.
2 Zetter, B. R. 1998. Angiogenesis and tumor metastasis. *Annu. Rev. Med.* 49:407.
3 Folkman, J. 2002. Role of angiogenesis in tumor growth and metastasis. *Semin. Oncol.* 29:15.
4 Takahashi, Y., Cleary, K. R., Mai, M., Kitadai, Y., Bucana, C. D. and Ellis, L. M. 1966. Significance of vessel count and vascular endothelial growth factor and its receptor (KDR) in intestinal-type gastric cancer. *Clin. Cancer Res.* 2:1679.
5 George, D. J., Halabi, S., Shepard, T. F. *et al.* 2001. Prognostic significance of plasma vascular endothelial growth factor levels in patients with hormone-refractory prostate cancer treated on cancer and leukemia group B 9480. *Clin. Cancer Res.* 7:1932.
6 Rofstad, E. K. and Halsør, E. F. 2000. Vascular endothelial growth factor, interleukin 8, platelet-derived endothelial cell growth factor, and basic fibroblast growth factor promote angiogenesis and metastasis in human melanoma xenografts. *Cancer Res.* 60:4932.
7 Ferrara, N. 2004. Vascular endothelial growth factor as a target for anticancer therapy. *Oncologist* 9:2.
8 Carrato, A., Gallego-Plazas, J. and Guillen-Ponce, C. 2006. Anti-VEGF therapy: a new approach to colorectal cancer therapy. *Expert Rev. Anticancer Ther.* 6:1385.
9 Fong, G. H., Rossant, J., Gertsenstein, M. and Breitman, M. L. 1995. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature* 376:66.
10 Skobe, M., Rockwell, P., Goldstein, N., Vosseler, S. and Fusening, N. E. 1997. Halting angiogenesis suppresses carcinoma cell invasion. *Nat. Med.* 3:1222.
11 Jain, R. K., Duda, D. G., Clark, J. W. and Loeffler, J. S. 2006. Lessons from phase III clinical trials of anti-VEGF therapy for cancer. *Nat. Clin. Pract. Oncol.* 3:24.
12 Holash, J., Davis, S., Papadopoulos, N. *et al.* 2002. VEGF-Trap: a VEGF blocker with potent antitumor effects. *Proc. Natl Acad. Sci. USA* 99:11393.
13 Presta, L. G., Chen, H., O'Connor, S. J. *et al.* 1997. Humanization of an anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res.* 57:4593.
14 Brekken, R. A., Overholser, J. P., Stastny, V. A., Waltenberger, J., Minna, J. D. and Thorpe, P. E. 2000. Selective inhibition of vascular endothelial growth factor (VEGF) receptor 2 (KDR/Flk-1) activity by a monoclonal anti-VEGF antibody blocks tumor growth in mice. *Cancer Res.* 60:5117.
15 Zondor, S. D. and Medina, P. J. 2004. Bevacizumab: an angiogenesis inhibitor with efficacy in colorectal and other malignancies. *Ann. Pharmacother.* 38:1258.
16 Riely, G. J. and Miller, V. A. 2007. Vascular endothelial growth factor trap in non small cell lung cancer. *Clin. Cancer Res.* 13:4623s.
17 Grandinetti, C. A. and Goldspiel, B. R. 2007. Sorafenib and sunitinib: novel targeted therapies for renal cell cancer. *Pharmacotherapy* 27:1125.
18 Kazatchkine, M. D. and Kaveri, S. V. 2001. Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. *N. Engl. J. Med.* 345:747.
19 Dalakas, M. C., Illa, I., Dambrosia, J. M. *et al.* 1993. A controlled trial of high-dose intravenous immune globulin infusions as treatment for dermatomyositis. *N. Engl. J. Med.* 329:1993.
20 Krause, I., Wu, R., Sherer, Y., Patanik, M., Peter, J. B. and Shoenfeld, Y. 2002. *In vitro* antiviral and antibacterial activity of commercial intravenous immunoglobulin preparations—a potential role for adjuvant intravenous immunoglobulin therapy in infectious diseases. *Transfus. Med.* 12:133.
21 Blank, M., Asherson, R. A., Cervera, R. and Shoenfeld, Y. 2004. Antiphospholipid syndrome infectious origin. *J. Clin. Immunol.* 24:12.

22 Merimsky, O., Meller, I., Inba, M., BarYehuda, S., Shoenfeld, Y. and Fishman, P. 2002. Possible role for IVIg in the treatment of soft tissue sarcoma: a clinical case and an experimental model. *Int. J. Oncol.* 20:839.
23 Murie-Fernández, M., Gurpide, A., de la Cruz, S. and de Castro, P. 2006. Total remission of thymus carcinoma after treatment with intravenous immunoglobulin. *Clin. Transl. Oncol.* 8:697.
24 Shoenfeld, Y. and Fishman, P. 1999. Gamma-globulin inhibits tumor spread in mice. *Int. Immunol.* 11:1247.
25 Fishman, P., Bar-Yehuda, S. and Shoenfeld, Y. 2002. IVIg to prevent tumor metastases. *Int. J. Oncol.* 21:875.
26 Sherer, Y., Levy, Y. and Shoenfeld, Y. 2002. IVIG in autoimmunity and cancer—efficacy versus safety. *Expert Opin. Drug Saf.* 1:153.
27 Shoenfeld, Y., Levy, Y. and Fishman, P. 2001. Shrinkage of melanoma metastases following high dose intravenous immunoglobulin treatment. *Isr. Med. Assoc. J.* 3:698.
28 Carmeli, Y., Mevorach, D., Kaminski, N. and Raz, E. 1994. Regression of Kaposi's sarcoma after intravenous immunoglobulin treatment for polymyositis. *Cancer* 73:2859.
29 Besa, E. C. and Klumpe, D. 1992. Prophylactic immune globulin in chronic lymphocytic leukemia. *N. Engl. J. Med.* 326:139.
30 Molica, S., Musto, P., Chiurazzi, F. *et al.* 1996. Prophylaxis against infections with low-dose intravenous immunoglobulins (IVIG) in chronic lymphocytic leukemia. Results of a crossover study. *Haematologica* 81:121.
31 Damianovich, M., Solomon, A. S., Blank, M. and Shoenfeld, Y. 2007. Attenuation of colon carcinoma tumor spread by intravenous immunoglobulin (IVIg). *Ann. N. Y. Acad. Sci.* 1110:567.
32 Sapir, T. and Shoenfeld, Y. 2005. Uncovering the hidden potential of intravenous immunoglobulin as an anticancer therapy. *Clin. Rev. Allergy Immunol.* 29:307.
33 Shapiro, S., Shoenfeld, Y., Gilburd, B., Sobel, E. and Lahat, N. 2002. Intravenous gamma globulin inhibits the production of matrix metalloproteinase-9 in macrophages. *Cancer* 95:2032.
34 Yoon, J. S., Kim, H. H., Han, J. W., Lee, Y. and Lee, J. S. 2006. Effect of intravenous immunoglobulin and methylprednisolone on human umbilical vein endothelial cells *in vitro*. *Immunobiol.* 211:351.
35 Couffinhal, T., Silver, M., Zheng, L. P., Kearney, M., Witzenbichler, B. and Isner, J. M. 1998. Mouse model of angiogenesis. *Am. J. Pathol.* 152:1667.
36 Iimuro, S., Shindo, T., Moriyama, N. *et al.* 2004. Angiogenic effects of adrenomedullin in ischemia and tumor growth. *Circ. Res.* 95:415.
37 Cao, R., Bråkenhielm, E., Pawliuk, R. *et al.* 2003. Angiogenic synergism, vascular stability and improvement of hind-limb ischemia by a combination of PDGF-BB and FGF-2. *Nat. Med.* 9:604.
38 Shoenfeld, Y. and Krause, I. 2004. IVIG for autoimmune, fibrosis, and malignant conditions: our experience with 200 patients. *J. Clin. Immunol.* 24:107.
39 Orbach, H., Katz, U., Sherer, Y. and Shoenfeld, Y. 2005. Intravenous immunoglobulin: adverse effects and safe administration. *Clin. Rev. Allergy Immunol.* 29:173.
40 Nakatani, K., Takeshita, S., Tsujimoto, H. and Sekine, I. 2002. Intravenous immunoglobulin (IVIG) preparations induce apoptosis in TNF-alpha-stimulated endothelial cells via a mitochondria-dependent pathway. *Clin. Exp. Immunol.* 127:445.
41 Xu, C., Poirier, B., Van Huyen, J. P. *et al.* 1998. Modulation of endothelial cell function by normal polyspecific human intravenous immunoglobulins: a possible mechanism of action in vascular diseases. *Am. J. Pathol.* 153:1257.
42 Takeshita, S., Zheng, L. P., Brogi, E. *et al.* 1994. Therapeutic angiogenesis. A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hindlimb model. *J. Clin. Investig.* 93:662.
43 Blank, M., Nur, I., Toub, O., Maor, A. and Shoenfeld, Y. 2005. Toward molecular targeting with specific intravenous immunoglobulin preparation. *Clin. Rev. Allergy Immunol.* 29:213.
44 Blank, M., Anafi, L., Zandman-Goddard, G. *et al.* 2007. The efficacy of specific IVIG anti-idiotypic Abs in antiphospholipid syndrome (APS): trophoblast invasiveness and APS animal model. *Int. Immunol.* 19:857.
45 Shoenfeld, Y., Rauova, L., Gilburd, B. *et al.* 2002. Efficacy of IVIG affinity-purified anti-double-stranded DNA anti-idiotypic Abs in the treatment of an experimental murine model of systemic lupus erythematosus. *Int. Immunol.* 14:1303.
46 Heil, M., Wagner, S. and Schaper, W. 2004. Arterial regeneration by collateral artery growth (arteriogenesis). *Drug Discov. Today Dis. Models* 1:265.
47 Carvalho, J. F., Blank, M. and Shoenfeld, Y. 2007. Vascular endothelial growth factor (VEGF) in autoimmune diseases. *J. Clin. Immunol.* 27:246.