VEGF–nitric oxide reciprocal regulation

To the editor — In a recent issue, Tsurimi *et al.* demonstrated that balloon angioplasty of rat arteries results in the induction of vascular endothelial growth factor (VEGF), the selective endothelial cell mitogen[1]. They also showed that sodium nitroprusside, a nitric oxide (NO) donor, inhibits protein kinase C (PKC)-induced VEGF upregulation. We have observed a biphasic regulation of VEGF expression by NO-donors. Induction of inducible nitric oxide synthase (iNOS) gene was seen with low concentrations of NO donor whereas inhibition of iNOS was seen with high concentrations of the NO donor. Increased NO release from endothelial cell by the VEGF isoform VEGF165 has also been shown[2] (see also Dembinska-Kiec, A. *et al.* Nitric oxide synthase (iNOS) and vascular endothelial growth factor (VEGF) gene expression in experimental restenosis. 66th Congress of the European Atherosclerotic Society, Florence, Italy, July 1996). Thus an interesting reciprocal relation between VEGF and NO is suggested.

VEGF exists in different isoforms, all being the result of alternative splicing of precursor mRNA. We have observed that in rat arteries, balloon angioplasty induces the expression of VEGF164 and VEGF188 (equivalents to human VEGF165 and VEGF189) mRNA, while VEGF120 was also present in control vessels (manuscript submitted). Upregulation of VEGF was delayed in comparison to the induction of iNOS. These *in vivo* studies reflect the course of iNOS and VEGF gene expression in vascular smooth muscle cells (VSMC) *in vitro:* iNOS mRNA was induced one hour after coincubation with IL-1β while VEGF gene expression was delayed up to 12-24 hours (manuscript submitted). Our preliminary studies on patients undergoing coronary angioplasty have shown that the peak of VEGF165 protein release occurs 48 hours after intervention, but is not accompanied by the increase in NOx concentration in blood (manuscript in preparation).

From clinical experience it is, however, evident that protective mechanisms induced after angioplasty are not sufficient in patients with lipid disorders, perhaps because modified LDL inhibits both iNOS and NO, as well as the induction of GTP cyclohydrolase I (manuscript submitted), the key enzyme for synthesis of tetrahydroblopterin — the cofactor necessary for NOS dimerization and change of O_2· generation to NO-generating activity. We have also demonstrated that ox-LDL inhibits IL-1β induced VEGF gene expression in rat VSMC.

Thus, the reciprocal relation between VEGF and NO may be disrupted in dyslipidemic patients, promoting the development of restenosis. If so, it will be important to normalize patients' lipid levels prior to angioplasty.

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Heparan sulfate and viral tropism

To the editor — The recent identification of highly sulfated heparan sulfate (HS) as the initial receptor for serotype 2 of dengue virus by Chen *et al.*[1] adds another virus to the list of those using HS as their initial receptor. It also raises questions concerning the specificity of the binding interaction between virus and cell surface HS, particularly given that virtually all living cells have HS on their surface and that HS is strongly negative in charge, and as such would favor electrostatic interactions with a plethora of proteins. A key aspect of the possible relation between HS binding and viral tropism was addressed by Putnak *et al.* in an accompanying *News & Views*[2] article — does the diversity in amount and structure of HS on different cells correlate with the efficiency of virus infection?

Similar issues have been addressed with respect to alphaherpesviruses. The two serotypes of herpes simplex virus, that is, type 1 (HSV-1) and 2 (HSV-2), which show predominant tropism for oral and genital tissue, respectively, both target HS as their initial receptor[3]. Competition experiments for virus binding to host cells have indicated type-specific differences in requirements of HSV-1 and HSV-2 for specific sulfate groups of heparin/HS (ref. 4). Furthermore, experiments carried out with pseudorabies virus, a related herpes virus infecting pigs, revealed somewhat different viral preferences as regards sulfate groups involved[5]. In addition, recent results using the HSV-1 attachment glycoprotein C has allowed the identification of structural motifs within cell surface HS that are different from other identified protein-binding HS sequences, suggesting that the interaction between the viral protein and HS is specific[6].

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