Xenogeneic endothelium: a cancer therapy?

To the editor:

I was very interested in the paper of Wei *et al.* entitled "Immunotherapy of tumors with xenogeneic endothelial cells as a vaccine"[1], in which they describe the successful application of xenogeneic endothelia for tumor prevention and therapy in mice. Nevertheless, some issues merit further discussion.

Wei *et al.* immunized mice with human endothelial lysates and analyzed the sera of the immunized mice using western blots. They found two major bands located at 220 kDa and 30 kDa [**AU: Do you mean 130kD? Wei *et al*indicate it is 130 kDa, not 30 kDa**], which they considered to be VEGFR-2 and α_v integrin (in fact, α_v integrin should be 150 kDa), respectively, as well as several weak bands (see figure on p. 1164 of their paper). However, when using whole cells as antigens, regardless of cell type or species of origin, the immunized animals will elicit a variety of antibodies to the many macromolecules on the cell membrane and in the cytoplasm and nucleus, including major histocompatibility complex antigens, receptors, cytoskeleton proteins and signal transduction molecules. Because all of these proteins can be immunogens, the immunized animals should produce antibodies against most, if not all, of them. In the experiments of Wei *et al.*, however, the mouse lymphocytes responded to very few cellular proteins.

VEGFR-2 is expressed on hematopoietic progenitor cells[2] and in the kidney, particularly in the glomerulus[3]. Integrin α_v is widely expressed on blood cells, macrophages, neutrophils, platelets[4], osteoclasts[5] and smooth muscle cells[4] and in the kidney[6], lung[7] and pituitary gland[8]. When Wei *et al.* adoptively transferred immunoglobulins from immunized mice to nonimmunized mice, antibodies to VEGFR-2 and α_v integrin would be expected to trigger disastrous sequelae such as platelet aggregation and glomerulonephritis in the recipients. Human cancer patients treated with several angiogenesis inhibitors, including antibody to VEGFR-2 [**AU: provide reference of primary research paper for this statement or remove**], were reported to experience thrombosis, hypertension and proteinuria, implying the involvement of platelets and kidneys[2]. In contrast, Wei *et al.*, report that they observed no deposition of immunoglobulins in the kidney endothelium [**AU: OK AS EDITED?**].

Wei *et al.* also synthesized several peptide fragments of α_v integrin (amino acids 330–384 and 545–579) and VEGFR-2 (239–273), and observed inhibited tumor growth after administering these peptides to tumor-bearing mice. Integrins consist of two subunits, the α- and β-chains. The α_v chain combines with one of five β-chains (β_1, β_3, β_5, β_6 or β_8) to form a heterodimer with a ligand binding site at the subunit interface. The signal resulting from ligand engagement is transmitted into cells by the β-chain. The antibody to integrin α_v used by Wei *et al.* is not conformation-specific and interacts only with the α-chain. As such, how did the antibody exert a biological effect if it could not react with the subunit interface of the heterodimer? In addition, a ligand (or an antibody that mimics it) can act as an antagonist or an agonist when it binds to an integrin. Immunization with the above-mentioned peptides of VEGFR-2 and α_v integrin would produce polyclonal antibodies that would react to different epitopes [**AU: EPITOPES OF THE INTEGRIN?**], and would not be predicted to cause only inhibitory effects, as observed by Wei *et al.* Moreover, because immunization with endothelia should induce antibodies against many cellular antigens, the polyclonal antibodies against VEGFR-2 and α_v integrin would be expected to account for only a very small percentage of the entire pool of antibodies. If this were the case, how could antibodies against VEGFR-2 and α_v integrin have such potent antitumor effects?

Lastly, in this study tumor size was expressed in terms of diameter. Because subcutaneous tumors, especially large ones, show an irregular oval rather than a spherical shape, it is unclear to us how Wei *et al.* measured tumor size. [**AU: PLEASE PROVIDE 1 TO 2 CONCLUDING SENTENCES.**]

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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Wei [AU: ARE THERE OTHER AUTHORS?] replies:

We thank Dr. Lusheng for his interest in our work. With regard to his first concern, to our knowledge there is no published evidence to support the view that animals immunized with whole cells as antigens should produce antibodies directed against most, if not all, of the proteins in those cells. In contrast, it has been reported that animals challenged with xenogeneic cells produce antibodies against some, but not most, of the cellular proteins[9–11]. Other cancer-cell vaccines elicit antibodies to only a single antigen[12] [**AU: Support statement with more than one reference if reference 12 pertains to only one vaccine.**]. Whether or not the molecules in whole cells can trigger the production of antibodies may depend on a variety of factors [**AU: CLARIFY WHAT THESE FACTORS ARE**]. We would also like to point out that α_v integrin has been identified as 130 kDa (ref. 13). [**AU: PLEASE NOTE THIS SENTENCE MAY BE REMOVED.**]

Lusheng's second concern is related to the potential side effects of targeting VEGFR-2 or α_v integrin. In fact, there are no reports of such side effects as thrombosis, hypertension or proteinuria in cancer patients treated with antibody to VEGFR-2. [**AU: INDICATE IN WHAT SETTING THESE SIDE EFFECTS ARE REPORTED AND ADD REFERENCE.**] Our findings are compatible with other reports in which no major toxicity was observed in mice treated with antibodies to VEGFR-2 or α_v integrin, or with a vaccine based on VEGFR-2 (refs. 14–16), even though these molecules may be expressed at low levels in some normal tissues. Clinical trial [**AU: TRIAL OR TRIALS?**] with anti-

body to the α_v integrin [**AU: OK?**] also resulted in little or no toxicity [**AU: Provide reference.**]

Third, in accordance with our observations, others have also shown that antibody to α_v integrin alone can inhibit tumor angiogenesis and migration of cancer cells[15,17] [**AU: edit ok?**], and that some antibodies against the nonbinding domain [**AU: please clarify what you mean by 'nonbinding domain'**] can allosterically block integrin function[17]. The high potency of the polyclonal serum in our study might result from targeting to multiple epitopes. This suggestion is supported by observations that the polyclonal antibodies obtained by immunization with xenogeneic endothelial cells show potent inhibition of endothelial cell proliferation, induction of endothelial cell apoptosis and abrogation of tumor growth, without major toxicity[9].

Finally, two perpendicular **diameter**s of each tumor were measured with calipers. Tumor size can be expressed in diameter, as reported by others[18].

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