

Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand

To the editor—Exciting results reporting that blockade of the CD40/CD40 ligand pathway by monoclonal antibody against CD40 ligand led to long-term acceptance of renal allografts in monkeys were recently reported in *Nature Medicine*¹ and elsewhere². Our own group has previously reported that mixed chimerism and renal allograft tolerance are achieved in monkeys conditioned with a multi-modality peritransplant regimen and without long-term maintenance immunosuppression^{3,4}. In an effort to simplify this therapeutic regimen, we tested a monoclonal antibody against CD40 ligand (American Type Culture Collection catalog number 5C8.33) in this protocol. In the first animals treated, we encountered an unusually high incidence of thromboembolic complications, which we thought worthy of reporting.

Using our standard regimen (non-myeloablative total body irradiation, thymic irradiation, anti-thymocyte globulin, donor bone marrow infusion and a 1-month course of cyclosporine), we found no thromboembolic complications in more than 50 animals. In contrast, after adding 20 mg/kg of monoclonal antibody against CD40 ligand to the regimen on days 0 and 2, we observed four thromboembolic complications in nine recipients. These included two renal artery thromboses, one renal vein thrombosis and one superior mesenteric artery thrombosis. In subsequent animals, the addition of 100 units/kg heparin immediately before the antibody (days 0 and 2) reduced the incidence of thrombotic complications to just two (one renal artery, one renal vein) in ten recipients. We then tried additional heparin treatments (100 units/kg, days 0,1,2,3) preceded by vigorous postoperative hydration. None of the five animals tested after this change have developed thrombotic complications. These observations may be relevant to ongoing clinical trials of monoclonal antibody against CD40 ligand (humanized version of 5C8) in which some thromboembolic complications have been reported (Vincent, J. *Biogen News* www.prnewswire.com, 11/2/99).

CD40 ligand was originally identified

on activated CD4⁺ T cells⁵, later on stimulated mast cells and basophils⁶ and most recently on activated platelets *in vitro* and *in vivo* on platelets in the process of thrombus formation⁷. CD40 is constitutively expressed on the vascular endothelium of various organs, and its ligation can upregulate adhesion molecules such as E-selectin, VCAM-1 and ICAM-1. Ligation through CD40 has also been reported to upregulate tissue factor expression on endothelial cells⁸. CD40 ligand expression has also been seen *in vivo* on activated platelets by examining fresh thrombi formed during vessel injury. In all fresh thrombi analyzed, CD40 ligand was expressed on a large proportion of platelets in areas in which densely packed platelets had not yet formed an amorphous mass and on platelets directly adhering to the vessel endothelium. These results may be relevant to the mechanism of a possible increased risk factor for thromboembolism induced by monoclonal antibody against CD40 ligand. Our results indicate that the administration of heparin in conjunction with monoclonal antibody against CD40 ligand can reduce the incidence of thromboembolic complications.

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Kirk and Harlan reply—Kawai and colleagues report a considerable number of thromboembolic events in monkeys after administration of a mouse monoclonal antibody against human CD154. This group's extensive experience with the monkey kidney allograft model, coupled with their unprecedented run of thrombotic complications, does suggest that something is awry, perhaps that the antibody preparation used in their experiments is prothrombotic. Indeed, a link between CD154:CD40 and coagulation makes great teleological sense. Nevertheless, what makes sense and what is fact are often very different. However appealing, given the

reported presence of CD154 on platelets and endothelia, we believe it premature to definitively conclude that the results discussed in this letter are epitope-specific.

Thromboembolic complications have been found after the initial use of many antibodies in humans and monkeys, and have largely disappeared with the use of refined antibody purification processes. Biogen's early clinical trials have been marred by thromboembolic events, indicating that humanized antibody against human CD154 induces thrombosis. These complications have been publicly disclosed and the trials have been halted pending additional pre-clinical evaluation. However, trials (admittedly different in design) using an antibody against human CD154 produced by IDEC Pharmaceuticals have proceeded so far without reported thromboembolic events.

Agents interfering with the CD154:CD40 pathway obviously powerfully influence the immune system and will no doubt have collateral effects. Potential effects, such as the ones presented here, must be scrutinized methodically and then corroborated by rigorous prospective investigation. Only then can important clues derived from anecdotes be accepted as fact.

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