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Antibody affinity maturation and respiratory syncytial virus disease

To the Editor:

The mouse immunization experiments reported in Delgado et al.[1] support the hypothesis that failure to elicit affinity-matured, neutralizing antibodies contributed to disease enhancement after immunization of children with a formalin-inactivated respiratory syncytial virus vaccine candidate (FI-RSV) in the 1960s. However, the authors' assertion that lack of protection by FI-RSV was "not due to alterations caused by formalin but instead to low antibody avidity for protective epitopes" is not justified by the data presented. On the contrary, Figure 3h of their paper presents data showing that neutralizing epitopes are largely absent from FI-RSV, presumably as a result of formalin inactivation or another insult during vaccine preparation. Their conclusion that antibody avidity alone was responsible for disease enhancement predicts that disease enhancement by FI-RSV could be overcome by stimulating affinity maturation with a Toll-like receptor (TLR) agonist. However, they did not report this key test of their proposed explanation for disease enhancement by FI-RSV in the paper. Delgado et al. do show enhanced protective efficacy of ultraviolet light–inactivated RSV upon formulation with TLR agonists, and other investigators have reported that formulating FI-RSV with monophosphoryl lipid A, a TLR4 agonist, reduces vaccine-induced immunopathology in immunized and challenged cotton rats[2]. The observations by Delgado et al.[1] and in the literature indicate that a protective RSV vaccine must both present neutralizing epitopes and elicit affinity-matured antibodies recognizing those epitopes.

It stands to reason that vaccine-mediated disease enhancement can occur only if a vaccine fails to elicit high-affinity neutralizing antibodies that prevent infection. However, contrary to the authors'[1] statement that "this study explains why the inactivated RSV vaccine did not protect the children and subsequently led to severe disease," their data do not explain why an RSV vaccine that fails to block infection actually enhances subsequent disease. TLR agonists drive more than just affinity maturation. They also enhance neutralizing antibody titers and breadth, promote isotype switching and alter the balance of T helper type 1 and T helper type 2 responses. Thus, these agonists might block disease enhancement by multiple mechanisms. The requirement for an RSV vaccine to elicit high-affinity neutralizing antibodies and the utility of TLR agonists are points well taken, but future vaccines must also present native RSV structures.

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Delgado et al. reply:

Shaw et al.[1] state that our observations indicate that a protective RSV vaccine must elicit affinity-matured neutralizing antibodies for enhanced respiratory disease (ERD) not to occur[2]. We agree with this statement. We also agree that, ideally, vaccines should present native respiratory syncytial virus (RSV) structures[2], although we think that modifications that do not affect the generation of neutralizing antibodies would be acceptable.

In addition, Shaw et al.[1] state that our paper blames ERD on the lack of "antibody avidity alone." This statement simplifies our observations. A widely accepted paradigm to explain ERD for decades ascribed the disease solely to formalin disruption of protective epitopes[3–5]. As we showed in our paper, these epitopes are still recognized by a formalin-inactivated RSV (FI-RSV)-elicited germline antibody[2]. Yet we subsequently showed that eliciting maturation of FI-RSV antibody would shift the response from native toward formalin-modified epitopes[2]. This process, which did not occur in the 1960s when the vaccine failed to elicit maturation, alters recognition of protective areas and explains our decision to conduct experiments using ultraviolet light–inactivated RSV instead of FI-RSV.

The common denominator for all nonreplicating vaccines that prime for ERD is the generation of low-affinity antibodies[6,7]. Thus, it is crucial to emphasize that there will be no safe nonreplicating RSV vaccine for infants in the absence of appropriate adjuvants. Certain vaccines may pose additional challenges, and we did not—at any point in our study—support the use of FI-RSV. We merely challenged the widespread belief that the problem with ERD is just a matter of epitope disruption, which could be solved using other methods for virus inactivation[2].

Shaw et al.[1] cite a study in which FI-RSV was formulated with a Toll-like receptor 4 (TLR4) agonist and reduced ERD symptoms[8] but fail to mention that RSV challenge shortly after immunization encountered transient protection (probably attributable to steric hindrance) in other nonreplicating RSV vaccine formulations[7,9]. So we conclude that formalin should never be used again to inactivate RSV for vaccine development.

Finally, there is a small but key difference between the statement quoted in the correspondence from Shaw et al.[1] ("…subsequently led to severe disease") and our statement in the abstract ("…did not protect the children and consequently led to severe disease")[2]. We showed that sera from mice immunized with ultraviolet light–inactivated RSV plus TLR agonists protected FI-RSV–immunized mice lacking antibodies from ERD after RSV challenge. In other words, as a consequence of antibody-mediated protection in these adoptive transfer experiments, and independent from

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TLR effects on T helper or other cells, ERD did not occur.

In summary, FI-RSV failed to protect primarily as a result of poor avidity, as germline antibodies continued to recognize protective epitopes. Moreover, specifically maturing FI-RSV–specific antibody would not have solved the problem. Last, no nonreplicating vaccine against RSV will be safe for infants if it fails to elicit affinity maturation.

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Will integrin inhibitors have proangiogenic effects in the clinic?

To the Editor:
In a comprehensive analysis, Reynolds *et al.*[1] recently reported that RGD-mimetic agents such as cilengitide may, under certain experimental conditions, promote rather than inhibit angiogenesis. They accordingly express their reservations regarding the clinical exploration of such agents in human patients with cancer.

On the basis of promising phase 2 data[2,3], cilengitide in combination with temozolomide-based radiochemotherapy is currently being explored in a phase 3 registration trial for newly diagnosed glioblastoma with O^6-methylguanine methyltransferase (*MGMT*) promoter methylation (CENTRIC trial, European Organisation for Research and Treatment of Cancer 26071–22072). This new paradigm of seeking approval for a first-in-class agent in a molecularly defined subpopulation of individuals with glioblastoma was based on the observation that the apparent clinical benefit derived from cilengitide in the phase 2 trial was prominent only in this patient population[3]. Do the proangiogenic preclinical data of Reynolds *et al.*[1] raise serious concerns regarding the potential for paradoxical effects of cilengitide in individuals with glioma *in vivo*? We believe that this may not be the case.

First, the clinical importance of the tumor models used by Reynolds *et al.*[1] may be questioned. Although the major target disease of the current clinical development of cilengitide is glioblastoma, no glioma model was studied.

Second, *in vitro* analyses suggest that there are multiple actions of cilengitide that mediate a clinical benefit in glioblastoma, including direct cytolytic effects on tumor cells, cytolytic effects on endothelial cells and inhibition of cell adhesion, migration and invasion[4]. Although the functional consequences of the interactions of cilengitide with its target integrins are probably complex in the context of glioma biology, the overall net effect in the clinic seems to be growth inhibitory rather than growth promoting[2].

Third, in the current clinical setting, cilengitide is used in combination with chemotherapy and radiotherapy, again on the basis of preclinical data showing strong sensitization to radiotherapy in rodent glioma models[5].

Fourth, pulse treatment as used in the clinical trials did not result in adverse effects in any of the models studied by Reynolds *et al.*[1]. In fact, the scheduling claimed to be tumor growth–promoting in their study[1] is not used in humans.

Fifth, cilengitide used at flat doses of 2,000 mg twice weekly results in peak plasma cilengitide concentrations of >200 μM, which, by orders of magnitude, exceed the concentrations shown by Reynolds *et al.*[1] to promote angiogenesis. In fact, simulations based on population pharmacokinetic models show that concentrations in the angiogenesis-promoting range (0.2–20 nM)[1] are not reached in 75% of patients treated with biweekly intravenous infusions of 2,000 mg cilengitide (J. Grevel (Merck Serono), personal communication). Micromolar concentrations of cilengitide have also been measured in the tumor tissue of patients with glioma exposed to the drug before surgery for recurrent disease[6]. Admittedly, the extent of blood-brain and blood-tumor barrier penetration of cilengitide in humans with glioma remains uncertain, and it remains uncertain whether potentially proangiogenic concentrations of cilengitide may be operational at least transiently in the tumor tissue.

Finally, although Reynolds *et al.*[1] suggest that cilengitide mediates angiogenesis by enhancing the effect of vascular endothelial-derived growth factor (VEGF), the striking neuroradiological responses to cilengitide seen in some individuals with glioblastoma[7,8] morphologically closely resemble the effects of VEGF-antagonizing agents such as bevacizumab[9]. On the basis of these considerations, we acknowledge that Reynolds *et al.*[1] have assembled an interesting and unexpected set of data in preclinical models. In fact, a paradoxical proangiogenic effect of cilengitide may be operative in certain settings and contribute to an antitumor effect of cilengitide in combination with radiotherapy or chemotherapy. This consideration relates to the vascular normalization effect of antiangiogenic agents, which we have proposed to underlie the preferential clinical benefit apparently seen in glioblastoma patients with *MGMT* promoter methylation[2]. The clinical importance, however, of the complex effects of cilengitide reported by Reynolds *et al.*[1] as well as by Alghisi *et al.*[10] can be assessed only in appropriately designed clinical trials.

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