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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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturemedicine/

1. Delgado, M.F. *et al. Nat. Med.* **15**, 34–41 (2009).
2. Boukhvalova, M.S. *et al. Vaccine* **24**, 5027–5035 (2006).

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