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In Response

Dear Sir:

The Letter to the Editor by Monath[1] regarding our study of co-administration of yellow fever (YF) 17D vaccine with human Ig[2] raises interesting points. The study evaluated the effect of Ig co-administration on YF 17D viremia and immune responses, and in doing so collected evidence regarding a hypothesis to potentially explain an increase in reported YF 17D vaccine-related adverse events (AEs). Before 1996 in the pre-hepatitis A virus (HAV) vaccine era, YF-specific antibodies in Ig given to travelers as HAV prophylaxis at the time of their YF 17D vaccination may have incidentally acted to reduce YF 17D viremia and consequent AEs.

The study objectives were to assess the effect of co-administered Ig, relative to saline control, on the proportions and magnitudes of YF 17D viremia, antibody response, T cell activation, and plasma cytokines. Persons were administered available contemporary Ig mimicking its historical administration in travel clinics for prevention of HAV: 1) intramuscular (IM) by 1.5-inch needle and syringe to the upper outer quadrant of the buttock; and 2) during a clinic visit when 17D vaccine was also administered. The study found that co-administered Ig, relative to saline control, did not modify 17D viremia, 17D-specific antibody response, T cell activation, or plasma cytokine levels. Those results argued against the underlying hypothesis. An alternate hypothesis for the increase in 17D adverse events is enhanced awareness, surveillance and reporting of YF 17D AEs as called for by the Centers for Disease Control and Prevention.[3] Furthermore, our study enrolled young healthy adults, whereas travel clinics administer YF 17D vaccine to a wider variety of the population, including older persons in whom the incidence of YF 17D AEs is greater.[4,5]

We agree that there could have been antibody or viral titer differences in historical Ig or YF 17D reagents, respectively. The 2006–2007 YF Ig study necessarily used available contemporary commercial Ig and 17D vaccine, and different results could have been obtained for persons administered Ig or vaccine that differed intrinsically, quantitatively, or qualitatively. Methods used for production or quantitation of Ig or vaccine may differ in different eras, or as Monath points out, for Ig the pool of serum donors may have changed. To address that concern, before the study, we tested 30 lots of Ig acquired through the Food and Drug Administration, for neutralizing antibody against YF by 50% plaque-reduction neutralization test ($PRNT_{50}$). The lots were from 1990–2003 and had concentrations of 5% to 16.5%. In all 30 lots, neutralizing antibody titers ranged from 1:160–1:2,560 (median = 1:640) and were sustained over time (Edupuganti S, unpublished data) (Figure 1). The figure not only indicates sufficiently high titers of protective antibody in all lots but also lot-to-lot variability in measured titers. Similar results were observed when $\log_{10}$ neutralizing index (LNI) assays were used; median LNI values across all lots over time were 2.91 (range = 1.61–4.13). A PRNT titer ≥ 20 or an LNI > 0.7 is protective against infection against yellow fever.[6]

Although Ig from 1990 through 2003 contained high levels of protective antibody to yellow fever virus, making our original hypothesis plausible, Monath points out that antibody levels at that time may have been even higher than the amount given to patients tested in our study, which might explain our negative findings. In addition, in our study, we reported that at day 7 after Ig and vaccine administration, there was no serum antibody detectable in PRNTs (Figure 1).[2] Detectable PRNT titer may not capture all antibody-mediated anti-viral activity in the vaccinated person because antibody that binds virus *in vivo* may also act through a number of Fc-mediated non-neutralizing functions not detected by PRNT, including antibody-dependent cell-mediated cytotoxicity, complement activity, and antibody-dependent cell-mediated virus inhibition.[7–10]

The dose of Ig in the study was that recommended by the manufacturer for HAV prophylaxis (0.06 mL/kg). The Ig was co-administered with YF 17D during a single clinic visit, again presumably as would have occurred for most travelers. Whereas IM injection in the deltoid is typically performed with a 5/8–1-inch needle and syringe, the YF Ig study nurses administered Ig in the gluteal muscle, presumably as it occurred in most travel clinics before 1996, to the upper outer buttocks quadrant into gluteal muscle with a longer 1.5-inch needle and syringe, consistent with published immunization and nursing guidelines for normal and overweight body mass indexes (BMIs).[11,12] As stated in the Materials and Methods,[2] participants received 2–3 injections of Ig or saline depending on their weight (maximum of 2 mL/injection).

Monath postulates that Ig could have been delivered subcutaneously because of obesity. Effectiveness of IM injections are determined by BMI, sex, and thickness of subcutaneous fat at the injection site, among other factors. In the YF Ig study, sex was well balanced in both groups because randomization was stratified by sex and race. The mean BMI of our entire study cohort was 25.3 kg/m^2; in the saline versus Ig groups BMI did not differ (25.5 kg/m^2 and 25.1 kg/m^2, respectively; $P = 0.72$). Likewise, participants in the overweight (25–29.9 kg/m^2) or obese (≥ 30 kg/m^2) BMI categories were distributed equally across Ig and saline groups with 18 (45%) of 40 in each group in these categories. For Ig versus saline groups, 5 (12.5%) versus 3 (7.5%) were obese. Thus, sex and BMI, factors that may influence subcutaneous fat thickness and IM injection success, were equal in the two study groups.

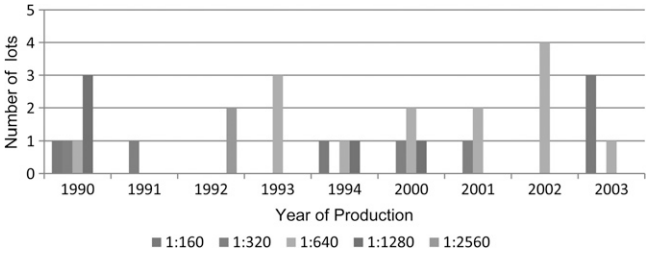

FIGURE 1. Neutralizing antibodies against yellow fever in lots of immune globulin, 1990–2003.

We do not believe Ig administration factors differed significantly from historical methods or impacted the results or conclusion. For ≈90% of our study participants who had normal or overweight BMIs, the needle length we used should have been adequate per guidelines. However, Ig with higher 17D-specific antibody could have produced a different result and conclusion. Our study as performed had a negative result that failed to provide support, and thereby argues against the underlying hypothesis, as stated in the abstract and final paragraph.[2] We agree that this negative result cannot disprove the hypothesis.

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