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Letter to the Editor

Evidence of Serological Cross-Reactivity between Genotype 1 and Genotype 3 Erythrovirus Infections

Candotti and colleagues (1) reported the prevalence of a third strain of human erythrovirus, genotype 3 (V9), in the Ghanaian population and, in part, concluded that a genotype 1 (B19)-based assay failed to detect genotype 3 immunoglobulin G (IgG) in 38.5% of Ghanaian samples containing genotype 3 antibodies. We disagree with this conclusion for the following reasons. (i) No details are furnished as to the expression system used for V9 VP1 production, the conformational status of the protein (denatured or native structure), or the purity or the concentration of the genotype 3 VP1 protein used for microplate coating. (ii) The number of specimens used to establish the cutoff of the V9 VP1 enzyme immunoassay (EIA) was extremely small ($n = 4$). (iii) The high degree of homology among capsid proteins of genotypes 1 through 3 would suggest that antibodies raised against one capsid type would be cross-reactive with the others (3, 7).

With respect to the first point, it is interesting that all specimens, seropositive in the genotype 1 VP2 EIA, were also positive in the genotype 3 "in-house" VP1 EIA (1), thus confirming that the genotype 1 assay could actually detect antibodies produced as a result of either genotype 1 or genotype 3 infection. This is supported by the fact that the seronegative specimens (genotype 1 EIA) were also negative by the genotype 3 EIA. An apparent discrepancy occurred only with the genotype 3 EIA, whereby certain specimens that were seroreactive via the genotype 3 EIA were negative when tested with the genotype 1 EIA. The absence of critical experimental information regarding VP1 production, purification, and immunoassay procedures cast doubt over the conclusions drawn by the authors with respect to genotype 3 immunoassay performance. Many erythrovirus VP1 purification procedures involve protein denaturation, thus destroying many of the diagnostically relevant conformational epitopes (5, 6, 8). It is well established, in genotype 1 erythrovirus infections, that IgG directed against linear epitopes of VP1 gradually declines postinfection but is maintained against conformational epitopes of VP1 and VP2 (4, 5, 6, 8). Therefore, if the VP1 used by Candotti and coworkers was denatured, then the assay would detect only antibodies against linear epitopes and would not identify all past erythrovirus infections. As the genotype 1 EIA used in this study utilizes capsid VP2, it can detect antibodies against conformational epitopes, thereby conferring high sensitivity of detection. Candotti and colleagues directly compared an EIA which uses genotype 3 VP1 structural protein, of unclear pedigree, to one that has been extensively validated (2) and employs genotype 1 VP2 structural protein.

The small panel of only four negative specimens used to determine the cutoff may increase the risk of obtaining false positives in the genotype 3 EIA. Indeed, it is notable that every specimen that was reactive only in the genotype 3 EIA had a significantly lower level of reactivity than those that were reactive in both the genotype 1 and genotype 3 EIAs. Thus, to verify these results, we suggest that an alternative method, such as an immunoblot, should have been employed.

It is worthy to note also the high degree of homology of the VP2 capsid proteins of genotypes 1 through 3, which would be expected to result in a high level of immunological cross-reactivity between these viruses (7). In fact, a previous study showed that a genotype 1 VP2-specific IgM EIA was capable of detecting both genotype 2 and genotype 3 viral infections (7). In addition, a genotype 3 immune response was initially detected by counterimmunoelectrophoresis with a genotype 1 virus-specific serum (7). Serological screening by enzyme-linked immunosorbent assay using genotype 3 recombinant VP2 found that the results were 100% concordant with those obtained by an enzyme-linked immunosorbent assay which uses genotype 1 VP2 as the antigen (3). This outcome indicates significant serological cross-reactivity against the genotype 1 and genotype 3 VP2 protein, making it an excellent protein for use as a diagnostic antigen. Conversely, as there is a greater degree of variation in the VP1 unique region between the three genotypes at the amino acid level, in particular, in one of the major neutralization epitopes, the use of VP1 alone would have been more appropriate to determine if there was a differential genotype-specific antibody response.

It is important to acknowledge that in the DNA-negative cohort from Ghana (1), the genotype 1-based assay was capable of detecting erythrovirus-specific IgG in 70% (107 of 153) of those tested. This rate is the average seroprevalence rate for the adult population (5, 9) and would imply that this assay was capable of detecting all genotype 3 infections if genotype 3 erythrovirus is prevalent in Ghana.

Finally, it should be noted that the genotype 1 VP2-based EIA is the only immunoassay capable of detecting erythrovirus IgG to have been cleared by the U.S. Food and Drug Administration and, as such, has been subject to extensive public validation (2). We submit that a more robust study is needed to confirm whether antibodies against all three erythrovirus genotypes cannot be detected using EIAs based on the capsid VP2 protein of genotype 1. The information, as presented by Candotti and coworkers, does not support this contention.

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Authors' Reply

We are grateful to Drs. Corcoran and Doyle to extend the discussion regarding the utility of VP2 genotype 1-based assays to detect antibodies to human erythrovirus (HEV) genotype 3. They question the origin of the genotype 3 antigen (V9) we used and the specificity of our enzyme immunoassay and infer that if antibodies from genotype 1-infected individuals react well with genotype 3 VP2 protein, the reverse might be true. It is unfortunate that the authors did not concede that the two samples containing both genotype 3 viral DNA and specific antibodies were not reactive with the Biotrin assay.

As to the pedigree of the V9 antigen, it was obtained from Drs. K. Brown and E. D. Heegaard, who prepared it and showed that genotype 1 antibodies cross-reacted with this antigen (4). This antigen was purified capsids formed of both VP1 and VP2 proteins, presenting conformational as well as linear epitopes (4). The discussion is limited to the efficacy of VP2 genotype 1 antigen to detect antibodies raised by genotype 3 HEV infection, a situation not assessed in a population prior to our article (3). As we point out in the discussion, it is possible that the VP1/VP2 V9 antigen contained epitopes not present in genotype 1 VP2 (particularly, neutralizing) and that these epitopes, eliciting antibodies with a different timing of occurrence than antibodies to VP2 capsids, might have given the V9-based test a sensitivity advantage (3).

Using four negative controls to determine the cutoff of an indirect EIA is not unusual, provided the cutoff was defined with a greater number of negative samples. Since 100% concordance between genotype 1- and genotype 3-based assays for genotype 1 antibody detection was found, the specificity of Heegaard's assay was not contested for this type of antibodies. It would be unlikely that false-positive reactivity would be restricted to testing a genotype 3-infected population. The likelihood that the assay was equally specific irrespective of the prevalent genotype is fairly high.

The argument of expecting the antibody prevalence found in adult populations from Western countries, such as France, United Kingdom, the United States of America, and Denmark, to be similar to that in West Africa does not stand, considering the difference in the standards of hygiene between these geographical areas. It is well known that the prevalence of ubiquitous viruses, such as cytomegalovirus, varicella-zoster virus, and human herpesvirus 8 in sub-Saharan Africa is significantly higher than that in Northern countries (1, 2). A higher prevalence of antibodies to HEV, as shown in other parts of Africa (5) was therefore expected in Ghana.

Finally, we agree that more data and comparisons with assays differing from each other only by the genotype of the capture antigen need to be collected. We are preparing purified recombinant antigens of VP2, VP1/VP2, and VP1 unique regions of HEV genotypes 1 and 3. The genotype 3 antigens were obtained from a Ghanaian strain. Direct comparisons of EIAs on sera from areas where genotypes 1 and 3 are prevalent will be conducted with large numbers of samples with all the technical guarantees required by the Biotrin group.

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