Dengue virus (DENV) exists in both sylvatic and urban/endemic ecotypes (15), and the potential for emergence of sylvatic strains has become a focus of research. Recently Mota and Rico-Hesse (10) attempted to evaluate the pathogenic potential of viruses belonging to different genetic subgroups of DENV serotype 2 (DENV-2). Based on the viremia levels and erythema index profiles of one sylvatic genotype and three (Asian, American, and Indian) urban/endemic genotypes evaluated using the NOD-scid IL2rγnull humanized mouse model, the authors concluded that sylvatic DENV-2 viruses possess a reduced pathogenic potential compared to strains belonging to urban/endemic DENV-2 genotypes. However, these conclusions ignore both patterns in their own data and a wealth of published ex vivo, in vivo, and epidemiological evidence collected over the past 40 years.

First, Mota and Rico-Hesse (10) reported that in their mouse model, the sylvatic virus produced a peak virus titer, which is correlated with DENV disease in humans (8, 11, 18), that was significantly lower than that of the Asian genotype but higher than that of either the American or Indian genotypes. Second, the sylvatic virus caused significantly less erythema than viruses of any of the urban/endemic genotypes. However, other studies have concluded that the association of erythema with disease severity is not clear (2). Finally, thrombocytopenia, which is more directly pertinent to disease severity (1, 19), was as severe or more severe in mice infected with sylvatic DENV than in mice infected with the other DENV-2 genotypes. In sum, these data suggest that sylvatic DENV may have a potential to cause dengue disease that is equal to or greater than that of at least two established urban/endemic genotypes.

The results of Mota and Rico-Hesse (10) are consistent with previous ex vivo experiments utilizing monocyte-derived dendritic cells (moDCs) as a surrogate model of human infection. No consistent differences in the replication profiles between sylvatic and endemic strains (16).

The suggestion that sylvatic DENV viruses pose little risk to human health is also contradicted by several documented cases of sylvatic DENV-2 infection resulting in clinical illness indistinguishable from classic dengue fever (DF) (4, 9, 12, 14, 17). Even more compelling is a recent, severe dengue case caused by a sylvatic strain from southeast Asia, which underscores the potential of sylvatic strains to cause hemorrhagic manifestations in humans (3). All of this evidence and most of the publications cited above were ignored by Mota and Rico-Hesse (10).

Finally, Mota and Rico-Hesse (10) argue that reemergence into the urban transmission cycle by sylvatic DENV strains is unlikely for the following reasons: (i) sylvatic strains have not caused any outbreaks in West Africa, and (ii) their sylvan transmission foci are being eliminated due to human environmental disruption. While extensive environmental disruption has occurred throughout the tropics, there is strong, published evidence of continuing sylvatic DENV outbreaks as well as human and primate seroconversions in West Africa (4, 7, 17). Research sponsored by the Institut Pasteur de Dakar has documented multiple sylvatic amplification cycles occurring at roughly 8-year intervals since 1980 in West Africa (5, 6, 13, 14). The most recent amplification cycle with the isolation of sylvatic DENV-2 from mosquito collections and human infections was documented in 2008 (A. Sall, Institut Pasteur de Dakar Senegal, personal communication). The recent isolation of DENV-2 from a human patient infected in peninsular Malaysia and its close relationship to a sylvatic strain isolated nearby from a sentinel monkey in 1970 also indicate the undetected maintenance of sylvatic DENV in a zoonotic cycle in southeast Asia for nearly 4 decades (3). Thus, the assertion of Mota and Rico-Hesse (10) that sylvatic dengue virus foci have been eliminated is baseless.

REFERENCES


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Authors’ Reply

We welcome the opportunity to respond to the comments of Vasilakis et al. concerning our recent publication on a mouse model of dengue disease that mimics human signs. They specifically refer to our results concerning the West African or sylvatic genotype of serotype 2 dengue viruses. Most of their comments are misquotes or misinterpretation of our data and those of others, and they have failed to publish data directly supporting their hypothesis that epidemic viruses could originate from sylvatic cycles in Africa and Malaysia. Thus, the premise that sylvatic viruses pose a threat to public health is baseless. We believe it is more important to focus research on the dengue virus genetic variants that are causing massive epidemics, some with severe disease and mortality, rather than expending limited resources on an unsupported hypothesis.

First of all, we would like to clarify the nomenclature used here, since it refers to two different concepts in virus classification: our definitions refer to geographic origins of the virus strains that constitute the genotype, described in 1990 (7), while those used by Vasilakis et al., described 10 years later (12), refer to ecologic associations. Our lab was the first to publish genetic evidence for a separate genotype of dengue serotype 2 viruses that originated in West African countries, and most of these were isolated from mosquitoes and some primates. Only one virus of this genotype was isolated from a human, and the unequivocal clinical diagnosis of this case was malaria, not dengue (8). Only three more dengue virus serotype 2 isolates from humans have been classified as sylvatic, but no detailed clinical descriptions of these cases were given other than that the patients had fever (3). Thus, a total of 4 sylvatic serotype 2 viruses have been isolated from humans in 40 years of research in this field, and their clinical significance is questionable. (The citation of unpublished data by A. Sall on other isolates from humans obviously does not constitute evidence; the other papers they cite do not include clinical descriptions or virus isolation to genetically characterize the virus—this is necessary to distinguish them from introduced genotypes, as described below). In fact, we and many others have established that the viruses causing epidemics in African countries were imported from India, up until the 1980s (7), or from Southeast Asia after that (reviewed in reference 13). Thus, the potential for virus “spillover” from sylvatic cycles is minimal, and the evidence for further maintenance of these cycles is dwindling.

In fact, a senior researcher from the Institut Pasteur, J. Rodhain, was the first to make a statement to this effect, in a 1991 review (9), where he states, “The constant reduction in size of natural forests tends to make the original simian epidemiologic cycle somewhat of a relic which, at present, has practically no importance as a reservoir. It would be necessary to take it into consideration only if, before its disappearance, the inter-human cycle died out, for example as the result of vaccination—a prospect which appears far in the future at present.” Therefore, even researchers with far more field experience in the area than the authors of this letter (combined) have stated that the sylvatic cycles are not important epidemiologically.

With regard to the role of a sylvatic virus causing dengue hemorrhagic fever in one patient in Malaysia (2), this paper is severely lacking in virologic data and needs corroboration because there was no characterization of the virus isolated from the patient. The only evidence as to viral etiology was a comparison of nucleotide sequences obtained by enzymatic amplification from infected cell culture, via reverse transcription (RT)-PCR, and there were no antigenic (e.g., neutralization with poly- or monoclonal antibodies, to compare to other viruses) or phenotypic (e.g., plaque formation or immunofluorescence in infected cells) tests of a virus isolate. There is only one other isolate from a sylvatic cycle in Malaysia, isolated in 1970, and the “new” virus was shown to be more than 99.1% similar to it by Cardosa et al. (2). Thus, the classic virological tests mentioned above are critical to rule out cross-contamination by a laboratory strain, as has been reported several times in the literature (6) with the isolation of New Guinea C-like virus strains in sporadic cases in several countries around the world.

Concerning the data that Vasilakis et al. claim to have presented in their publications that demonstrate the virulence of sylvatic viruses for humans, these are nonexistent. It is impossible to use negative data from phenotypic assays (infection of mice transplanted with Huh-7 cells or growth rates in cell lines of nonhuman origin or human liver cancer) (10, 11) that cannot detect differences between wild-type viruses, as support for their hypothesis. In fact, in their 2007 report, Vasilakis et al.
show that the sylvatic viruses grow at lower rates than all others in primary human monocytes (11), thus supporting our previously published conclusions (4).

Vasilakis et al. also misquote or misinterpret our data in the recent paper (5); most of these are misinterpretations of clinical signs that are not statistically significant. We were the first to describe an animal model of disease that presents clinical signs of dengue as in humans, with viremia, erythema, thrombocytopenia, and fever (1). In our most recent article, where we compared 8 different low-passage viruses of serotype 2 in a new strain of humanized mice with respect to viremia, we stated, “Mice infected with virus strains of the Indian and West African genotypes showed the shortest viremia periods, with viremia falling to undetectable levels by days 16 and 14 p.i., respectively (Fig. 1C and D), and the Indian and American viruses showed the lowest levels of viremia (around 10^3 RNA copies/ml at peaks).” The latter were not “significantly lower” as Vasilakis et al. state. However, the sylvatic viruses did cause “significantly less erythema” but not significantly higher thrombocytopenia (Vasilakis et al. interpret our thrombocytopenia data as “severe or more severe in mice infected with sylvatic DENV”). Also, we mentioned that because of this shorter time span of viremia, the sylvatic viruses “seem to be at an evolutionary disadvantage, even more so than the American genotype viruses that have been displaced by the SE Asian viruses in many countries.” We did not assert “that sylvatic dengue foci have been eliminated” as stated by Vasilakis et al., but “there should be less concern because these viruses have not caused outbreaks in West Africa, and countries in that area have actually imported their epidemic viruses, while their sylvatic dengue foci are being eliminated by human environmental disruption.”

In summary, there is no evidence that sylvatic viruses have the potential to cause dengue epidemics, and we suggest that Vasilakis et al. place their research efforts on obtaining rigorous evidence for their “out-of-Africa” hypothesis. As we stated in our paper, our humanized mouse model of dengue fever continues to be the only source of data on virulence of wild-type dengue viruses, and the data from these animals suggest that sylvatic viruses are at an evolutionary disadvantage compared to other serotype 2 strains.

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