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Letter to the editor Open Access

Establishment of a non-lethal model of antibody-dependent enhancement of infection in A129 mice based on a non-mouse-adapted dengue virus strain

DEAR EDITOR,

Dengue fever (DF), caused by dengue virus (DENV) infection, is a highly prevalent mosquito-borne infectious disease. The development of antiviral drugs and vaccines relies on the clinical utility of dengue infection models, particularly those involving antibody-dependent enhancement (ADE). In this study, we co-incubated DENV-2 anti-prM with DENV-3 for 2 h, with subsequent infection of A129 mice to establish an ADE infection model. During the acute infection phase, viral nucleic acid copies in the liver, intestine, spleen, lung, and serum were significantly elevated in the ADE group compared to the DENV group. Pathological analysis revealed more pronounced damage to the liver, intestine, and lung in the ADE group, which also showed fewer monocytes and platelets but a greater number of lymphocytes and neutrophils. Fluorescence immunoassay confirmed that the expression levels of transforming growth factor-β (TGF-β) and tumor necrosis factor-α (TNF-α) in the liver were significantly higher in the ADE group than in the DENV group. Furthermore, $CD4^+$ T cells and $CD14^+$ neutrophils were significantly up-regulated in the ADE group. Collectively, these findings indicate the suitability of A129 mice for establishing ADE models.

At present, DENV infection is recognized as one of the most widely distributed insect-borne diseases, affecting more than 100 countries and threatening more than 2.5 billion people, thus posing a major public health challenge (Pierson & Diamond, 2020). The primary vectors responsible for DENV transmission are mosquitoes, especially *Aedes albopictus* and *A. aegypti* (Guzman et al., 2010). DENV infection presents in various clinical forms, including DF, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) (WHO, 1997). Severe cases of diagnosed disease are commonly associated with secondary or multiple infections (Shepard et al., 2016). It is widely accepted that ADE may play a role in the pathogenesis of severe DENV infection, although our understanding of this mechanism remains limited (Shepard et al., 2016). Establishing successful ADE infection models would be conducive to advancing our understanding of its underlying pathogenesis and provide valuable animal models for drug selection and vaccine safety evaluation. Previous studies have successfully established lethal infection models using DENV-2 (Zheng et al., 2020) and intraperitoneal injection of a non-mouse-adapted DENV-2 (D2Y98P) strain in AG129 mice (Tan et al., 2010). Thus far, ADE mouse models have primarily concentrated on AG129 and AG6 mice (Kayesh & Tsukiyama-Kohara, 2022), with few reports on the establishment of ADE models in A129 mice. In our study, we aimed to establish more stable and reproducible ADE models by co-incubating commercially available DENV-2 anti-prM with DENV-3 for 2 h, followed by infection in A129 mice. All animal experiments were approved by the Animal Ethics Committee of the Institute of Medical Biology, Chinese Academy of Medical Sciences (Kunming, China; Approval No. DWSP202110027).

To assess disease progression between the DENV and ADE groups, various parameters, including survival rate, body weight, and clinical scores (Supplementary Figure S1), were continuously monitored for 15 days post-infection. Compared to mice in the DENV and negative control (NC) groups, mice in the ADE group exhibited a rapid onset of clinical symptoms, including wrinkled appearance (9/9) and hunchback posture (4/9) on day 6, weight loss (9/9) on day 10, and different degrees of sluggishness (9/9) (Supplementary Figure S1). To determine viral load, the number of viral nucleic acid copies was measured on days 1, 4, and 7 after infection, with the highest number detected on day 4 (Figure 1A). In addition, on day 4 post-infection, the ADE group exhibited significantly higher levels of viral RNA in the serum, liver, intestine, spleen, and lung compared to the DENV group, with particularly elevated levels in the lung and spleen, as well as a significantly higher number of viral nucleic acid copies in the serum and multiple organs. On day 2 post-infection, whole blood samples were taken from the NC, DENV, and ADE groups to determine various hematological parameters, including platelet, monocyte, white blood cell, lymphocyte, and neutrophil counts, hematocrit and hemoglobin levels, and lymphocyte (LY%) and neutrophil percentages (NEUT%). Compared to the NC and DENV groups, the ADE group

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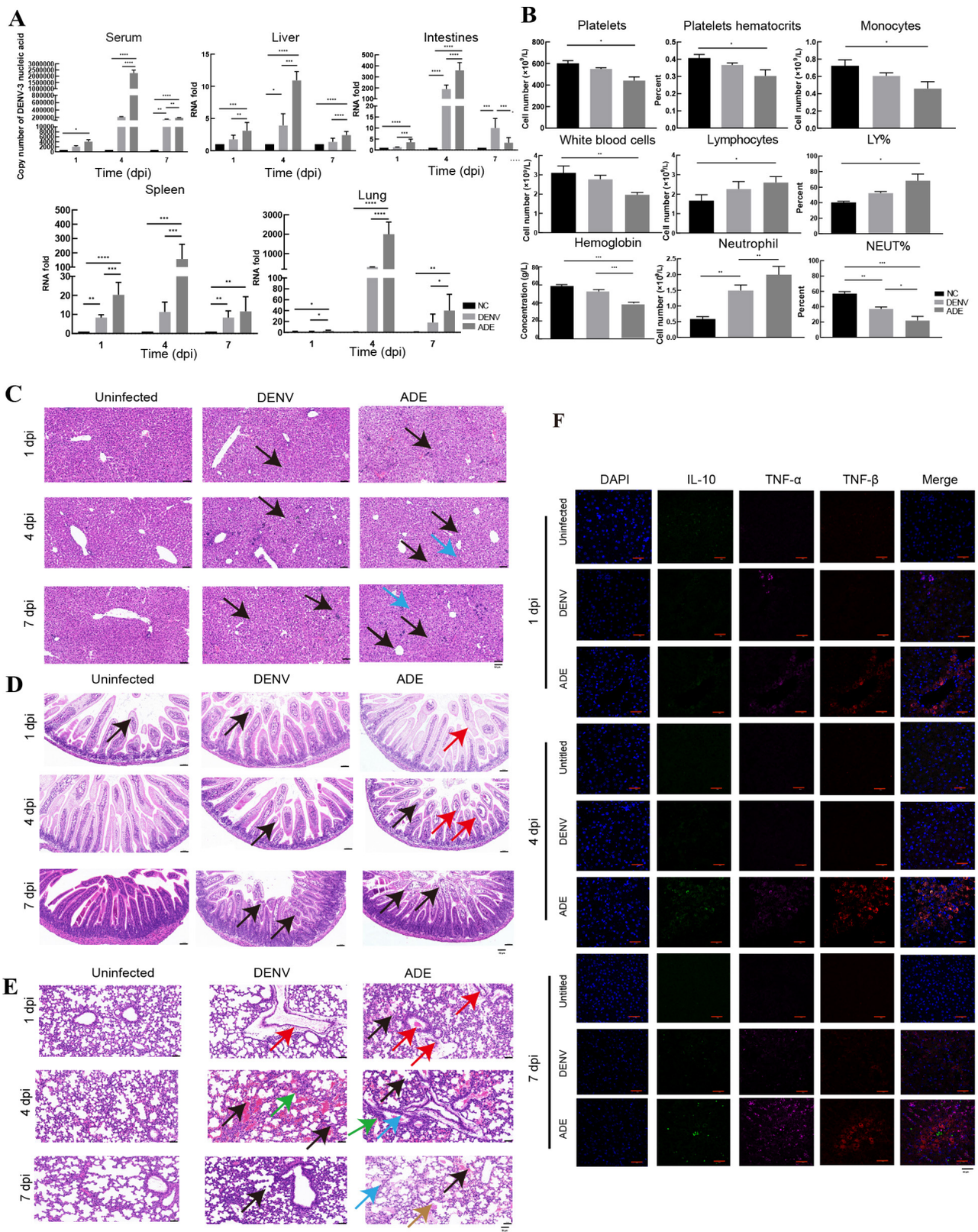

Figure 1 Establishment of a non-lethal model of DENV-3 and ADE infection in A129 mice

A: Viral replication kinetics and tissue tropism in DENV-3 and ADE infection in A129 mice. *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; ****: $P \leq 0.0001$, n=5 mice/group. B: Whole blood cell count in DENV-3 and ADE infection in A129 mice. *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$, n=5/group. C–E: Pathological changes in liver (C), intestine (D), and lung (E) tissues in DENV-3 and ADE infection in A129 mice (sections were observed under a light microscope at 200× magnification). The arrow points to the lesion. F: Expression levels of IL-10, TGF-β, and TNF-α in liver tissues in DENV-3 and ADE infection in A129 mice (blue represents DAPI staining. Green, pink, and red represent IL-10, TGF-β, and TNF-α staining, respectively. n=3/group). dpi: Days post infection.

demonstrated lower platelet, hematocrit, monocyte, white blood cell, and hemoglobin levels (Figure 1B). The decrease in platelets and hematocrits is similar to symptoms observed in critically ill patients (Malavige & Ogg, 2017). Pathological changes in the liver, intestine, and lung were observed in the NC, DENV and ADE models. On day 4 after infection, both the DENV and ADE groups exhibited mild vacuolar degeneration (black arrows) in the liver tissue, while the ADE group also showed a small number of lymphocytes in the hepatic lobules (blue arrow). By day 7 post-infection, mild vacuolar degeneration persisted in the liver tissue of both groups, while lymphocyte infiltration (blue arrow) was rarely observed in the hepatic lobules of the DENV group (Figure 1C). On day 4 post-infection, the mucosal epithelial cells in the large intestine of the ADE group gradually became looser and lightly stained (black arrow) and the villus epithelium detached from the lamina propria (red arrow). On day 7 after infection, the DENV group exhibited extensive degeneration of intestinal villus mucosal epithelial cells in the large intestine, as well as the presence of different-sized round vacuoles (black arrow) in the cytoplasm, while the ADE group showed numerous intestinal villi in the large intestine (Figure 1D). Lung tissue of the DENV group showed occasional, scattered lymphocyte infiltration around the blood vessels (red arrow) on day 1 post-infection, with minimal inflammatory cell infiltration (black arrow) and occasional acidophilic tissue fluid in the alveolar cavity (green arrow) by day 4. In the ADE group, lung tissue showed a small amount of inflammatory cell infiltration (black arrow), substantial bronchial bleeding (blue arrow), and excessive alveolar hemorrhage (green arrow). On day 7, the DENV group displayed a small amount of inflammatory cell infiltration in the lung tissue (black arrow), while the ADE group exhibited a large area of hemorrhage in the lung, several red cells (black arrow) in the alveoli and bronchi, and occasional protein fibers (brown arrow) in the alveoli, with local pulmonary interstitial edema and loosened and lightly stained alveolar wall (blue arrow) (Figure 1E). Taken together, the pathological damage to the liver, intestine, and lung was significantly more severe in the ADE group, consistent with the viral nucleic acid copy number findings (Figure 1A). To compare the expression levels of certain inflammatory cytokines in A129 mouse tissues between the DENV and ADE models, we performed immunofluorescence staining of the liver (Figure 1F), intestine (Supplementary Figure S2A), and lung (Supplementary Figure S2B). On days 1, 4, and 7 post-infection, the liver expression levels of TGF-β and TNF-α were significantly higher in the ADE group compared to the NC and DENV groups (Figure 1F), while interleukin 10 (IL-10) was highest on day 4 in the ADE group (Figure 1F). Additionally, the $CD4^+$ and $CD8^+$ T cell proportions differed significantly in the ADE group on days 3 and 5 after infection, with an elevation in $CD4^+$ T cells and a reduction in $CD8^+$ T cells (Supplementary Figure S3). Compared with the NC and DENV groups, $CD14^+$ neutrophils were significantly higher on days 3 and 5 post-infection, especially day 3, while $CD64^+$ neutrophils were significantly lower, especially on day 5 (Supplementary Figure S3).

Given the global increase in DENV cases, the development of drugs and vaccines for DENV infection remains of utmost importance. In this study, we successfully used commercially available DENV-2 anti-prM antibodies and DENV-3 to establish a stable and reproducible ADE model, which exhibited similar disease characteristics to AG129 mice (Sarathy et al., 2015). The ADE group displayed more severe organ damage and a higher viral load in the liver, intestine, and lung, as well as a markedly reduced number of platelets, similar to the outcome observed in patients with severe DENV infection (Malavige & Ogg, 2017). Both the DENV and ADE groups showed an imbalance in $CD4^+$ and $CD8^+$ T cells. Furthermore, the expression pattern of IL-10, a biomarker of disease progression in patients with severe DF infection (Tsai et al., 2013), in the intestine of the A129 infection model is consistent with clinical findings. In conclusion, we successfully established a non-lethal ADE mouse model that demonstrated clinical consistency with patients suffering from severe DENV infection. However, due to differences in genetic background and viral susceptibility between animals and humans, some clinical indicators in the A129 model may differ, thus requiring the continued development of more suitable models.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

H.W., M.W.L., and Q.M.S. designed the research. L.Z. performed the literature review. Y.P. and J.Y.C., and K.F. provided the study materials. H.W. and M.W.L. performed the experiments. H.W. and M.W.L. wrote the manuscript and analyzed the data. Q.M.S. performed article revision. All authors read and approved the final version of the manuscript.

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