PAF's fever pitch

By Tim Fulmer, Senior Writer

By drawing parallels between disease cascades, a Brazilian-led team has found that a sepsis target—platelet activating factor—may be a good intervention point for treating severe dengue fever. Targeting the proinflammatory mediator reduced the severity of dengue infection in mice,[1] and the group plans to have a platelet activating factor receptor inhibitor in the clinic by year end.

There are four serotypes of the mosquito-borne dengue virus, and neutralizing antibodies against one serotype don't protect against infection by the other three. Indeed, previous infection by any one serotype puts the patient at risk of severe dengue fever upon infection with a second dengue serotype. Severe dengue infection is characterized by extensive plasma leakage and hemorrhaging and can lead to hypotension, shock and death.

The Brazilian and Russian researchers noted that those features of severe disease resemble what happens in septic shock. As a result, the group hypothesized that the same or similar proinflammatory mediators might underlie both conditions. The researchers zeroed in on platelet activating factor (PAF), which is known to trigger systemic inflammation and vascular permeability in rodents.[2] Some dengue patients have also shown PAF upregulation.[3]

PAF-targeting compounds have been tested in the clinic for sepsis, but, like virtually every other sepsis therapeutic, did not show significant efficacy.

The logical step was to antagonize the PAF receptor (PAFR) in rodent models of dengue and look for reductions in vascular permeability and hemorrhaging. However, wild-type dengue virus replicates very poorly in mice, so the group's first priority was establishing a good rodent model of severe dengue infection.

The researchers generated a strain of dengue-2 (DEN-2) that was adapted to grow in murine tissue. Adult mice infected intraperitoneally with the strain showed hallmarks of severe dengue infection: thrombocytopenia, increased plasma leakage from the bloodstream, hypotension and hemorrhaging in the liver and lungs.

With a good model in hand, the researchers carried out genetic and pharmacological experiments to test the effects of inhibiting PAFR. Infected mice with *Pafr* knockout had significantly greater survival ($p<0.01$) and lower thrombocytopenia and plasma leakage ($p<0.01$) than infected wild-type littermates, although there were no differences in viral loads in the blood and spleen.

In wild-type mice infected with the DEN-2 adapted virus, administering the PAFR inhibitor UK-74,505 (modipafant) five days after infection significantly decreased thrombocytopenia and plasma leakage compared with administering vehicle ($p<0.01$). Moreover, the generic small molecule delayed lethality and increased the number of surviving mice.

Starting treatment seven days after infection also afforded partial protection. Treated mice also showed less hemorrhaging in liver tissue than controls.

The authors concluded that "therapeutic use of PAFR antagonists in humans may ameliorate manifestations of dengue and prevent evolution to severe disease." The findings were published in the *Proceedings of the National Academy of Sciences*, and the team was led by Mauro Teixeira, professor of biochemistry and immunology at **Federal University of Minas Gerais**.

Into mice or men

By year end, Teixeira and colleagues plan to begin a Phase I/II trial of UK-74,505 to treat dengue infection. Other researchers, however, wanted to see further proof of principle in models beyond the new one described in the *PNAS* paper.

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> ***—Pei-Yong Shi, Novartis Institute for Tropical Diseases***

"The PAFR inhibitor in the *PNAS* paper showed benefit against only a single mouse-adapted dengue virus strain," noted Pei-Yong Shi, senior unit head of dengue research at the **Novartis Institute for Tropical Diseases**. "The compound should now be validated using other available dengue models" to confirm that the anti-inflammatory effects are not unique to the adapted DEN-2 strain.

Indeed, adaptation of a dengue virus to allow it to grow in mice can lead to an overly attenuated strain that lacks the pathogenic characteristics of wild-type virus in humans.

Alternatives to using an adapted dengue strain include immunodeficient murine models that could be engrafted with human cells susceptible to infection by wild-type dengue virus.[4]

Nonhuman primate models of dengue infection are also an option worth considering, as they may more closely reflect the pathophysiology of human disease, said Mariano Garcia-Blanco. He is professor of molecular genetics, microbiology and medicine at the **Duke University School of Medicine** and professor at the **Duke University–National University of Singapore Graduate Medical School**.

Teixeira told *SciBX* his team "is now validating the *PNAS* findings using a second mouse-adapted strain of dengue-3 (DEN-3)." However, he said, they have no plans to look at primates.

"Because UK-74,505 appears safe in humans—as judged by the asthma clinical trials—a primate study would not, in our opinion, be useful," said Teixeira. "That will obviously need to be discussed with regulatory authorities of specific geographical regions where we plan to carry out human trials."

UK-74,505 has already shown safety in humans. The molecule was developed more than 15 years ago by **Pfizer Inc.** to treat asthma[5] but was never commercialized.

The team's planned Brazilian Phase I/II trial will enroll patients with both first-time and second-time dengue infection. Safety will be the main focus of the trial. Efficacy endpoints will include platelet levels and mean blood pressure.

Teixeira told *SciBX* the patent covering the use of UK-74,505 to treat asthma has expired and that he and his colleagues have filed for a patent covering the use of UK-74,505 and other PAFR antagonists to treat acute dengue infection. The findings are available for licensing.

Pfizer declined to comment on the status of the compound or the results in the *PNAS* paper.

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