Sneak peek at early course of bladder infection caused by widespread, understudied parasite

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Using standard tools of the molecular-biology trade and a new, much-improved animal model of a prevalent but poorly understood tropical parasitic disease called urogenital schistosomiasis, Stanford University School of Medicine researchers were able to obtain "snapshots" of shifting gene activity levels during the early, acute phase of what for most becomes a chronic bladder infection.

The findings, described in a study to be published online Nov. 29 in PLoS Neglected Tropical Diseases, could lead to new diagnostic and therapeutic approaches to urogenital schistosomiasis, a chronic disease that infects about 112 million people, almost entirely in developing countries and particularly in Africa, said Michael Hsieh, MD, PhD, assistant professor of urology and the study's senior author.

"Schistosomiasis is a huge global health problem and one of the most neglected of the neglected tropical diseases," said Hsieh. "Some 150,000 people die each year from urogenital-schistosomiasis-induced kidney failure. And this doesn't include deaths from bladder cancer or as a result of increased susceptibility to other infections, such as HIV, attributable to schistosomiasis-generated genital tract damage." Estimates that take into account chronic schistosomiasis' overall impact on quality of life place it on a par with other world-class health scourges such as malaria and tuberculosis.

Schistosomiasis is caused by a parasitic worm of the genus *Schistosoma*. Different *Schistosoma* species infect different organs, said Hsieh, whose research focuses on infection-induced bladder inflammation. *S. haematobium*, which is responsible for infection of the urogenital tract, is acquired by exposure to contaminated water. "The parasite larvae can smell fatty acids in our skin as well as detect motion. They're like smart bombs, equipped to find us."

After invading the skin and a pass through the circulatory system, the larvae mature to adulthood, lodge in blood vessels of the bladder wall and other pelvic organs and begin to lay eggs. A single worm can extend to several millimeters, lay hundreds of sand-grain-sized eggs every day and live for years to decades, Hsieh said.

In a study published recently in the British Medical Journal, John Ioannidis, MD, DSc, a Stanford professor of medicine, deplored the dearth of treatments for neglected tropical diseases including schistosomiasis. There are no vaccines for preventing it, and only a single drug, praziquantel, is approved by the World Health Organization for treating it. While cheap and relatively effective, it doesn't cure all infections. Plus it tastes terrible, which causes a surprisingly large number of people to stop taking it. Experts believe it's just a matter of time before the parasite develops resistance to praziquantel.

Despite a pressing need to come up with good anti-*S. haematobium* agents, almost all the existing medical literature is on other *Schistosoma* species. That's because until recently there were no practical animal models for *S. haematobium* infection. "We know next to nothing about the earliest molecular events in the bladder after eggs enter the tissue," said Hsieh. "When you try to naturally infect mice by simply exposing them to the parasite, the mice develop an infection in their liver or intestine instead of their bladder wall."

In March of this year, Hsieh and his colleagues reported, in the journal PLoS Pathogens, that they had solved this problem by injecting *S. haematobium* eggs directly into the bladder wall of ordinary laboratory mice. For the new PLoS-NTD study, Hsieh and his colleagues used this mouse model to synchronize the initiation of bladder infection by the parasite, thereby synchronizing the timing of post-infection changes that take place in bladder tissue so the researchers could better observe the parasitic infection's early trajectory. They injected about 3,000 parasite eggs into each of a few dozen lab mice and used microarray technology—a method of globally measuring the activity of virtually every gene in a tissue—to monitor thousands of genes' activity levels in bladder-wall tissue over the ensuing weeks.

The investigators set their microarray probe's sensitivity to flag any gene whose activation level either increased or decreased by a factor of two or more. They monitored thousands of genes whose activity levels were either amped up or tamped down at one, three and five weeks post-infection. These changes peaked at merely three weeks post-infection. "This is an important result," said Hsieh. "It shows that continuous waves of egg deposition, not just the initial schistosome infection, are what's generating chronic disease. So if you can kill the worms flat-out, you should be able to halt the cycle."

It would have been impossible to see this simply by observing patients, said Hsieh. "With the natural human infection, you never know when any given person is initially infected—unless it's that unfortunate American tourist that got infected while swimming in Lake Victoria on a weeklong Africa visit," he said.

The team also noted that these gene-activity changes fell into clusters. "Very soon after infection, we saw changes in activation levels in a cluster of cancer-associated genes. Likewise with another cluster of genes involved in fibrosis, or scarring, in the bladder. This confirms that

fibrosis kicks in very quickly," said Hsieh—a not unexpected result, as parasite eggs' burrowing causes significant bladder-wall damage.

On the other hand, within the first week there was a general suppression of genes associated with maintaining the integrity of the bladder wall. Inadequate production of this group of molecules would be expected to result in a leaky bladder wall, said Hsieh, and it probably did. "This happened at a time when the mice were shedding eggs in their urine. So it probably accounts for not only a lot of urinary symptoms but a significant aspect of the disease's transmission—the expulsion of eggs into the urine, which eventually contaminates another body of water," he said.

Finally, as has been observed in infections produced by other *Schistosoma*l species, the Stanford team noted signs of a shift in character of the immune response toward a state that it is oriented to tissue repair and to isolating pathogens—effectively attempting to enclose them in cement-like jails called granulomas—in order to prevent further tissue damage. (This mode of immune response may be best-suited to combating relatively large pathogens that would be likely to withstand an attempt by the immune system to kill them outright in a frenzy of inflammatory fury.)

Hsieh's group corroborated their microarray results at both intracellular and macroscopic levels with ultrasound, microscopic and molecular assessments. For instance, discernible evidence of scarring in bladder-wall tissue coincided with microarray readings indicating fibrosis-gene activation.

"Schistosomes have evolved with mammals for millions of years," said Hsieh. "Our shared evolutionary history makes our findings in mice likely to be relevant to humans. These observations may also have implications for fibrotic disease in general. Understanding fibrosis is

very important, because it is involved in cardiovascular disease, cancer and other diseases accounting for up to half of all deaths in the developed world."

The *S. haematobium* mouse model may also pinpoint biomarkers that enhance the detection and monitoring of incipient bladder cancer, a poorly understood disease.

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