Tuberculosis may lurk in bone marrow stem cells of infected patients, researchers say

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Tuberculosis is a devastating disease that kills nearly 2 million people worldwide each year. Although antibiotics exist that can ameliorate the symptoms, the courses of therapy last for months and don't completely eradicate the disease, which frequently recurs years or decades after the initial treatment.

Now, in a classic case of bench-to-bedside research, scientists at the Stanford University School of Medicine have discovered a possible reason for the disease's resistance: The ability of the tuberculosis bacteria to infiltrate and settle down in a particular class of stem cell in the bone marrow. By doing so, the bacteria take advantage of the body's own mechanisms of self-renewal.

"Cancer scientists have noted that self-renewing stem cells like these in the bone marrow have properties - such as natural drug resistance, infrequent division and a privileged immune status - that make them resistant to many types of treatment," said Dean Felsher, MD, PhD, professor of oncology and of pathology. "Now it turns out that this ancient organism, Mycobacterium tuberculosis, figured out a long time ago that, for the same reasons, these cells are ideal hosts to invade and in which to hide."

Not only did the scientists find genetic material from the bacteria inside the stem cells, they were also able to isolate active bacteria from the cells of human patients with tuberculosis who had undergone extensive treatment for the disease. The findings raise the possibility that other

infectious agents may employ similar "wolf-in-stem-cell-clothing" tactics. And, although any new human treatments are likely to still be years away, they suggest a new possible target in the fight against tuberculosis, which infects nearly 2.2 billion people worldwide.

"We now need to learn how the bacteria find and infect this tiny population of stem cells, and what triggers it to reactivate years or decades after successful treatment of the disease," said postdoctoral scholar Bikul Das, MBBS, PhD.

Felsher is a co-senior author of the study, which will be published online Jan. 30 in *Science Translational Medicine*. Das is the lead author. The research was conducted in collaboration with scientists from the Forsyth Institute in Cambridge, Mass.; the Hospital for Sick Children in Toronto; and several research groups in India.

The research focuses on a subset of stem cells in the bone marrow called mesenchymal stem cells. These cells are multipotent, meaning they can become several different types of specialized cells, including bone, fat and cartilage. Although the mesenchymal stem cells are most often found in the bone marrow, they are known to be able to migrate to sites in the lungs, where the tuberculosis bacteria thrive.

"Hematopoeitic cells, especially macrophages, have long been thought of as the primary intracellular niche for M. tuberculosis, even when the infection is present at a very low levels and the individual is asymptomatic," said Kevin Urdahl, MD, PhD, an assistant professor at Seattle Biomedical Research Institute, the country's largest independent organization devoted to the study of infectious diseases. Urdahl was not involved in the research. "However, this study shows that the bacteria also has the capacity to reside within mesenchymal stem cells, and may even persist in these cells after drug treatment. Although further studies will be needed to establish the relative importance of this niche during

latent infection, the immunoprivileged nature of the bone marrow and the ability of mesenchymal stem cells to express drug efflux pumps make this an intriguing possibility that could have important clinical implications."

Although tuberculosis is most commonly known as a disease of the lungs, it can infect many parts of the body, including the abdomen, bone, skin and brain. The respiratory form of the disease is spread through infectious particles aerosolized when an infected person coughs or sneezes. Many cell types have been found to harbor tuberculosis bacteria, but the location of the bacteria's primary (and highly successful dormant variant) hideout has remained unclear. However, Das noticed a clue during his years as a physician in India.

"Fifteen years ago, I was treating hundreds of tuberculosis cases," said Das. "At the time, we noticed we were finding tuberculosis bacteria in bone marrow biopsies that had been obtained from some of these patients for other reasons. This was a totally unexpected and accidental finding, but it gave me the idea that the bacteria could be infiltrating these cells."

To test his finding, Das, who came to Stanford as a postdoctoral scholar after completing a fellowship at the Hospital for Sick Children in Toronto, exposed bone marrow stem cells from healthy human donors to the tuberculosis bacteria. He found that not only did the bacteria infect the cells, but that they were also able to persist inside the cells for at least two weeks as they were maintained in culture. Upon closer investigation, he found that the bacteria preferentially infect mesenchymal stem cells expressing a cell surface marker called CD271 and that the viability of the bacteria in the cells decreased if the stem cells were stimulated to specialize, or differentiate, into other cell types.

Das next turned to a mouse model of dormant tuberculosis devised and

created by his colleagues in Cambridge. This model relies on a genetically modified strain of tuberculosis bacteria that can replicate only in the presence of a compound called streptomycin. In the absence of streptomycin, the bacteria remain dormant in the animal in a manner similar to that seen in treated human tuberculosis patients.

Together the researchers exposed laboratory mice to aerosolized particles of the modified bacteria. The mice became infected, and dormant bacteria were found in the CD271-expressing mesenchymal stem cells in the bone marrow of the animals six months after streptomycin withdrawal. When Das and his colleagues injected other mice with these tuberculosis-carrying stem cells, those animals went on to develop characteristic symptoms of the disease, including lung lesions called granulomas.

"These mesenchymal stem cells have never been implicated as a host for tuberculosis," said Felsher, "and they serve as a potential source for dormant disease. Moreover, these cells express drug-efflux pumps in their outer membranes that could make them resistant to anti-tuberculosis medications."

Finally, Das turned to collaborators in India to determine whether what happened in the mice reflected what happens in infected people. The researchers conducted a small clinical study in which bone marrow biopsies were collected from nine people who had undergone the complete course of anti-tuberculosis treatment and whose sputum, a mucus-like substance secreted into the airways of the respiratory tract, contained no detectable bacteria. In eight of the nine people, the researchers were able to detect bacterial DNA in the mesenchymal stem cells obtained from bone marrow; in two of these eight, they were able to isolate living bacteria.

"Not only is this strong evidence that the tuberculosis can remain

dormant in stem cells, but it shows that the living bacteria could be recovered from these cells after a long period of time," said Das. "It's also very suggestive of how the reactivation could be triggered: These stem cells are known to migrate to sites of injury or inflammation and begin dividing. So, migrating stem cells harboring dormant bacteria might reactivate the disease in the lung. Interestingly, I and other physicians treating patients with chronic obstructive pulmonary disease - which results in lung inflammation - have seen a strong correlation between COPD and tubercular relapse. It is possible that the tuberculosis relapse in COPD might involve the stem-cell mediated reactivation of a dormant tuberculosis infection."

In the future the scientists plan to focus on investigating the cellular mechanisms used by the tuberculosis bacteria to infect and persist in the mesenchymal stem cells, and how reactivation occurs on a molecular level. They're also interested in the possibility that tuberculosis might not be the only microbial bad boy that's learned how to exploit the stem cells' properties as a perfect hiding place.

"This could possibly be a more general paradigm," said Felsher. "Other infectious agents might use stem cells in a similar manner. We'd like to further characterize whether and how these stem cells provide a protective niche for other infectious agents."

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