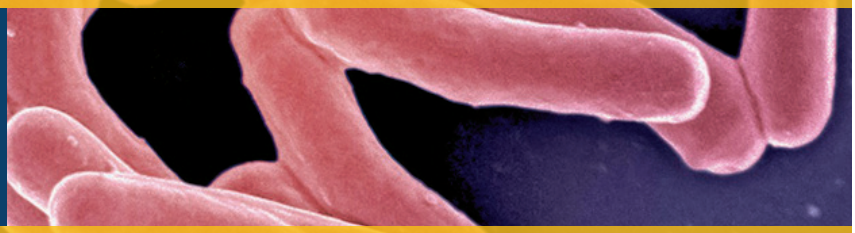
Me vs. TB: *Boosting the Immune System to Defeat an Ancient Adversary*



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Transformation of Original Research Article

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Abstract

Bacterial pathogens, such as ***Mycobacterium tuberculosis*** (TB), have plagued humans for centuries. During that time, it is estimated TB has caused over 1 billion deaths. Since their discovery, antibiotics have been the standard treatment for bacterial pathogens. However, overuse of antibiotics has resulted in drug-resistant strains of bacterial pathogens, including TB. Drug-resistant strains of TB do not respond to antibiotic treatment which has led scientists to investigate ways to strengthen the body's own immune system to fight TB. From reading cancer research, TB researchers learned that Host-Directed Therapies (HDTs) were successfully used to boost cancer patients' immune systems. For the first time in TB research, HDTs were investigated for effectiveness against drug-resistant TB. During in vitro studies, HDTs and antibiotics were tested alone and in combination for effectiveness to reduce the TB load in granulomas. The data outcomes are promising, indicating HDTs may be an important addition to TB treatment. Further research, including in vivo studies, are called for.

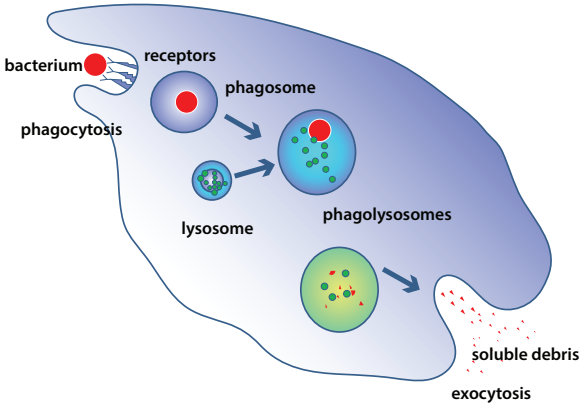
Background

In recorded human history, tuberculosis (TB) is the number one killer infectious disease, responsible for over an estimated 1 billion deaths globally. Today, the TB bacteria is the second leading cause of death due to an infectious disease, second only to COVID-19. Over the past two decades, the rate of TB infection has steadily declined by 2% each year. However, with the COVID pandemic, the rate of TB infection has increased by 3.6%. Without treatment, about 50% of those infected will die. However, with treatment, 85% of individuals infected with TB will be cured. But with all good news, there is usually some not so good news.

The treatments for TB include the antibiotics rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB). Treatment starts with a mixture of all four antibiotics for two months. After the first two months, PZA and EMB are removed. For the following four months, TB patients receive RIF and INH. All totaled, TB treatment can take six months. Although the cure rate is high, patients experience unpleasant

side-effects. These side-effects include nausea, persistent tingling in hands or feet, general weakness, and blurry or changed vision. Some of these side effects can be permanent.

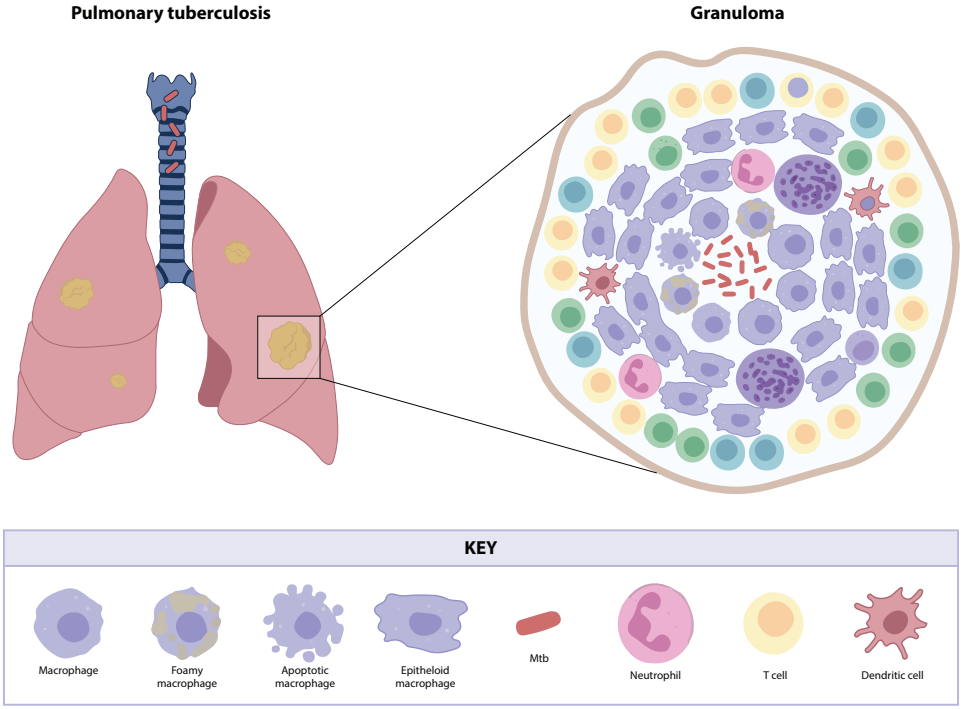
Figure 1. Phagocytosis



Despite the success of TB treatments, research is revealing an increase in drug-resistant strains of TB. Most TB bacteria that are drug resistant develop a resistance to one or both RIF and INH, making effective treatment challenging for doctors, but even more challenging for the infected patient. The increased rate of TB infection along with increased levels of drug-resistant TB are motivating scientists to find a better way to deal with this ancient adversary. When TB enters the lungs, the immune system sends specialized cells, called macrophages, to the infected areas. Macrophages engulf individual bacteria through a process called ***phagocytosis*** (fay-go-sigh-TOW-sis). During phagocytosis, the cellular membrane of the macrophage extends around the entire bacteria (Fig. 1). The macrophage membrane encases the bacteria, forming a phagosome (FAY-go-sohm), which is taken into the macrophage. Once inside the macrophage, cellular structures called ***lysosomes*** (LIE-so-sohms) fuse with the phagosome. Lysosomes contain enzymes. These digestive enzymes enter the phagosome and destroy the TB bacteria.

But sometimes the macrophages are overwhelmed by the number of TB bacteria. If macrophages are not able to kill all the TB bacteria, other immune cells are recruited and travel to the TB infected area where the immune cells surround the TB bacteria. The resulting structure is called a granuloma. Granulomas contain the bacteria and prevent further infection. However, granulomas do not kill all the bacteria and only contain the TB bacteria (Fig. 2).

Figure 2. Granuloma Structure Located in Lung Lobe



Host-Directed Therapies

Despite an 85% cure rate, the recent increase in TB cases is troubling. Antibiotics are the first line of treatment for bacterial infections, but the structure of granulomas makes it hard for antibiotics to reach and destroy the encapsulated TB bacteria. As a result, antibiotics lose their efficacy against TB bacteria. Thus, the presence of granulomas contributes to the lengthy treatment of TB. But granulomas are formed by the body's own immune response. This raises the question: how can scientists develop TB therapeutics when the body's own immune response to bacterial infection creates a structure that essentially protects the bacteria?

Host-directed therapies (HDTs) are treatments which have been shown to boost the body's own immune system against cancer. The most studied HDT is vitamin D. When used as part of cancer treatment, vitamin D has been shown to boost the body's immune system. It specifically increases the effectiveness of macrophages against cancer cells which reduces chemotherapy treatments. There is evidence that HDTs could also be effective to treat infectious diseases, like TB.

Death Pathways

Cells have a limited lifespan. There are several mechanisms which cause cell death, known as death pathways. Cell death is a normal process which is crucial to maintaining optimal body functions. Cell death pathways are the body's mechanisms to get rid of unneeded, old, or abnormal cells to make room for healthy, functioning cells. There are multiple cell death pathways used by macrophages as they encounter abnormal cells like those found in cancer or invading pathogens, like TB. Two of these cell death pathways are ***apoptosis*** (A-pop-TOH-sis) and ***necrosis*** (neh-CROW-sis).

When a macrophage encounters a pathogen, like TB, phagocytosis is triggered. The macrophage surrounds the TB bacteria and undergoes a self-destructive process – the necrosis cell death pathway. The macrophage "kills" itself and the TB within. In necrosis death pathway, the "deceased" macrophage bursts open releasing chemical signals that alert the immune system about the invading pathogen. The immune system responds by sending more macrophages. As each macrophage phagocytoses a TB bacterium, the necrosis death pathway repeats, causing an inflammatory response. In the short term, the inflammation caused by the release of macrophage contents is beneficial as it activates the immune system. But over time, this inflammatory response creates other health issues, like damaging the lung and inhibiting breathing. These health issues are not reversed even when antibiotics kill all the TB bacteria.

Apoptosis is a pre-programmed, normal cell death pathway. Each cell has a pre-programmed life span which is determined by a series of natural molecular steps. As cell death by apoptosis is a normal process, there is no need for an immune system response. To keep from triggering an immune system response, cells that undergo apoptosis do not release their contents. Their cell membranes do not break apart keeping the cell content contained as the dead cells are eliminated from the body.

When a macrophage phagocytoses a TB bacterium, the macrophage destroys the bacterium. But the presence of a pathogen shuts off the normal apoptosis cell death cycle and the necrosis cell death pathway is triggered. Initially, this works to the body's benefit. The cell membranes of the macrophage break apart, releasing its contents and alerting the immune system that a pathogen invader has been discovered. The immune system responds to the chemical signal by sending more macrophages and the cycle repeats, with

more macrophages absorbing TB bacteria and undergoing necrosis. The necrosis cell death pathway is great for alerting the immune system; however, if the TB is not killed before the macrophage breaks apart, the TB bacteria is released when the macrophage breaks apart and TB can infect other cells.

TB and Apoptosis

Through their research, scientists discovered TB bacterium shut off the apoptosis cell death pathway. With the apoptosis pathway disabled, the TB bacteria can replicate causing the cell to swell as more and more TB bacteria are produced within the infected cell. With the apoptosis pathway shut off, the cell membrane will rupture through necrosis, releasing TB bacteria to infect more cells and the disease progresses. If scientists could disrupt TB's ability to shut off apoptosis, then macrophages would undergo apoptosis instead of necrosis. In apoptosis the membrane does not rupture, and the cell, even when filled with TB bacteria, would be safely eliminated from the body.

Data from cancer research shows that specific HDTs induce apoptosis which reduces the duration of cancer treatment. Reducing the duration of treatment means patients' exposure to harsh side effects from cancer therapeutics is also reduced. Like cancer treatments, current TB therapeutics also have harsh, long-lasting side effects, which can become permanent. The parallels between cancer treatments and TB treatments inspired the TB researchers to shift their focus from medications and antibiotics to HDTs which specifically induce apoptosis. No one had ever investigated HDT-induced apoptosis to limit TB infection. The challenge was to find HDTs which could block TB bacteria's ability to disable the apoptosis cell death pathway. If possible, the membranes of any TB infected cells would stay intact, and as the cell undergoes apoptosis cell death pathway, the TB bacteria would be contained and eliminated from the body.

Targeting apoptosis with HDTs should control TB in multiple ways: limit TB growth within host macrophages and boost the immune system without triggering an inflammatory response. The scientists hypothesized HDTs would reduce TB growth within macrophages and within structurally complex granulomas.

METHODS

To test their hypothesis using in vitro methods, the scientists needed macrophages and granulomas. The macrophages were obtained from two sources: healthy human volunteers and laboratory mice, referred to as ***murine*** (mhur-REEN). Blood samples collected from human volunteers were cultured to grow human monocyte-derived macrophages (MDMs). Murine macrophage samples from bone marrow were collected and cultured. The murine samples are known as bone marrow-derived macrophages (BMDMs).

Granulomas and blood samples were also obtained from healthy human volunteers. Granulomas are complex structures which contain monocytes, macrophages, and lymphocytes. The blood samples contain the immune cells found in granulomas. Due to the complexity of granulomas and the multiple types of immune cells that can be found in granulomas, the scientists needed to generate granulomas with multiple cell types.

A strain of TB bacteria known to be resistant to current therapeutics was introduced to culture dishes, each containing the mixture of immune cells. Previous research showed that four days after introducing TB bacteria to cultured immune cells, granulomas would begin to aggregate or form. These granulomas are typically stable for up to 12 days. Knowing this, the researchers could pace their experiments for reliable results.

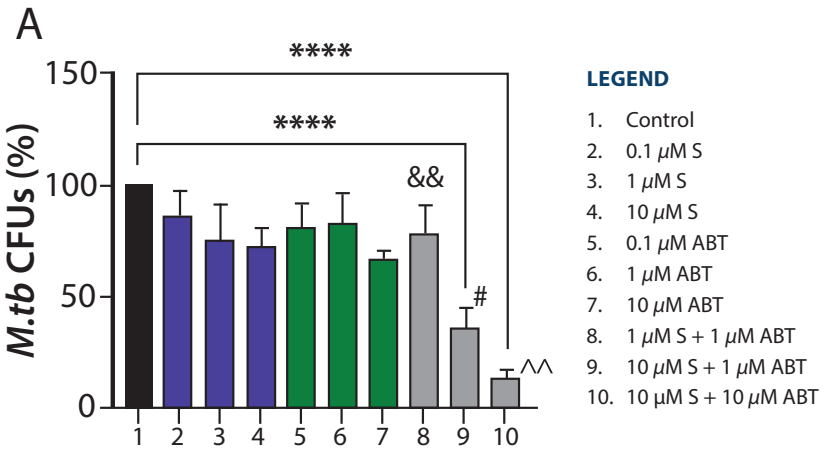
The scientists identified two proteins that are induced by TB infection and are important for TB growth in macrophages: myeloid cell leukemia 1 (MCL-1) and B-cell lymphoma 2 (BCL-2). Evaluating cancer research data, the scientists realized that MCL-1 and BCL-2 proteins inhibit or stop apoptosis. These two HDT inhibitor proteins are referred to as S63845 (S) and venetoclax/ABT-199 (ABT).

The scientists conducted in vitro tests on TB-infected MDMs, BMDMs, and granulomas to assess different combinations of the HDTs (S and ABT) on macrophages and granulomas containing TB bacteria. Some macrophages/granulomas were treated with current TB antibiotics, some with HDT's and antibiotics, while others received no treatment at all as a control.

RESULTS

TB infected MDMs were treated with the HDTs alone or in combination for a four-day period. After four days, the macrophages were lysed (broke open) and TB culture forming units (CFUs) were counted. Resulting data from this research indicated that MCL-1 inhibitors (Fig. 3, blue bars) reduced TB growth in human macrophages, as did BCL-2 inhibitors (Fig. 3, green bars). However, data from in vitro experiments show combining the HDT inhibitors S and ABT was significantly more effective than S or ABT alone at treating TB bacteria within macrophages (Fig. 3, grey bars).

Figure 3. MDMs infected with TB then treated with HDTs (see Legend).



NOTE: Colony Forming Units (CFUs)

HDTs are used with antibiotics, the amount of TB CFUs within macrophages is further reduced, more so than antibiotics or HDT alone. The complexity of granulomas presents an issue with conventional TB treatments. However, similar to macrophages, the scientists found that HDT with S + ABT significantly reduced TB even encapsulated within in vitro granulomas. (Fig. 5).

To evaluate how rapidly the HDTs could reduce TB bacteria, trials were conducted daily for up to four-days. The data show that after just one day the amount of TB CFUs in the control (untreated macrophages) were significantly higher than macrophages treated with a combination of HDT S + ABT. (Fig. 4).

The outcome of this research provides promising results for the addition of HDTs to TB treatments. When the proteins MCL-1 and BCL-2 are inhibited, TB growth is significantly limited in human and murine macrophages. Our research indicates when

Figure 4. CFU Comparison

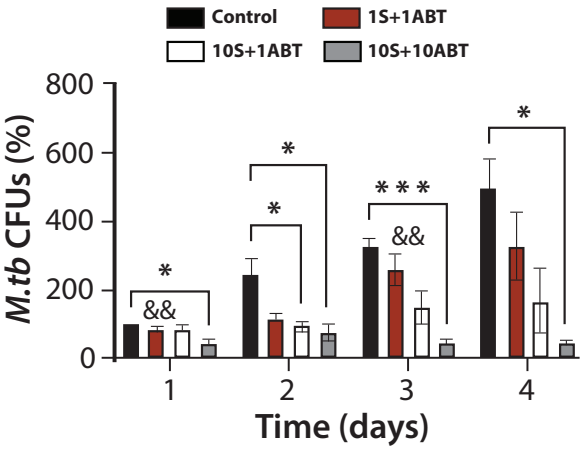
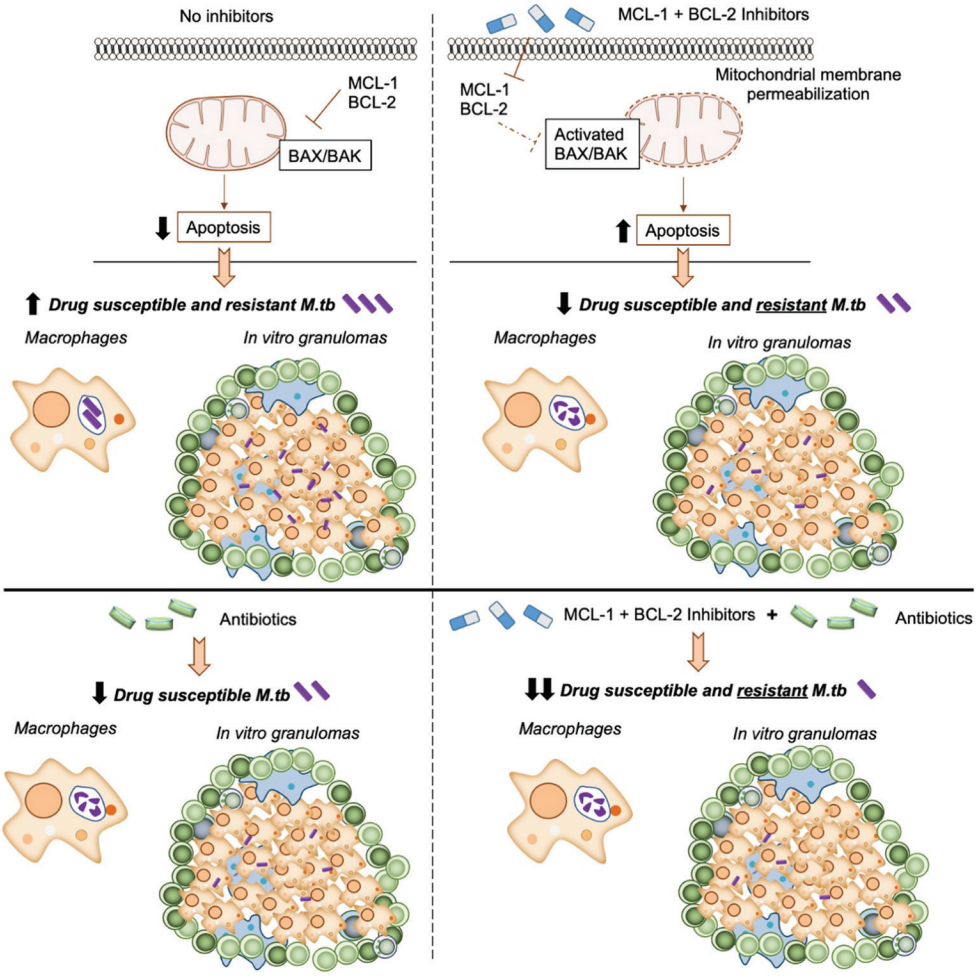


Figure 5. Comparison Between Treatment With and Without HDTs



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Conclusion

The HDTs in this study are already being studied in clinical trials for cancer. In fact, ABT is already FDA approved for treatment of some cancers. This study shows promising applications of these same HDTs from cancer research to limit TB infection. The addition of HDTs to antibiotic treatment may shorten TB treatment. By reducing the duration of treatment, the development of drug-resistant TB is also reduced. Perhaps most excitingly, by inducing apoptosis the HDTs should also reduce lung damage, which the antibiotics do not do.