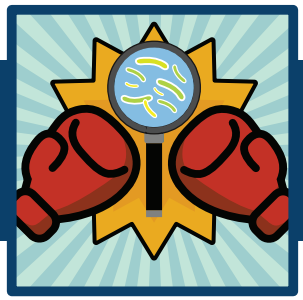
UNIT: TUBERCULOSIS

LESSON 3: ME VS. TB - BOOSTING THE IMMUNE SYSTEM TO DEFEAT AN ANCIENT ADVERSARY



TEXAS BIOMEDICAL RESEARCH INSTITUTE
HEALTH STARTS WITH SCIENCE

Lesson Objectives:

- Analyze the extrapolated data to determine the effectiveness of the TB treatment. (3A, 3D)
- Evaluate career connections applied to TB research. (3A, 3B, 3C)
- Interpret data to support which treatment most negatively impacts the progression of TB. (3A, 3C)
- Model components, from the lab to support staff, of TB research. (3B, 3D)

Lesson Support Information

The transformed article, *Me vs. TB*, provides students the opportunity to engage with current science research about an ancient pathogen that over the centuries has killed over a billion people. Tuberculosis is an ancient adversary that only infects humans. Tuberculosis has coevolved with humans. Over the centuries, TB has developed mechanisms to ensure its survival within humans. The discovery of antibiotics in 1910 and penicillin in 1923 finally provided humans tools to fight against pathogens, like TB. However, issues with antibiotics including overprescribing and not finishing antibiotic prescriptions led to drug-resistance. Until COVID, TB was the leading cause of death by a pathogen. Tuberculosis continues to be a threat to humans as cases of TB on a global scale have continued to increase for the first time in 20 years. This increase in cases has challenged the biomedical research community to investigate innovative ways to fight TB. Antibiotics are effective at treating TB; however, treatment can last four to six months with harmful side effects that can last a lifetime. From past research, scientists at Texas Biomedical Research Institute (Texas Biomed) knew the importance of the immune system in fighting TB but were searching for ways to fight TB from within. In other words, how can the immune system be fortified? These scientists examined research about other "invasive" conditions searching for protocols which effectively strengthened the immune system. One such research paper focused on cancer and the effect of Host-Directed Therapies (HDTs) on the immune system. The cancer researchers found adding HDTs, like Vitamin D, to cancer treatment boosted the immune system which increased the effectiveness of conventional treatments and shortened the duration of treatments. The Texas Biomed scientists decided to test the effect of HDTs on TB.

General Information

Tuberculosis (TB), ***Mycobacterium tuberculosis***, is a bacterium that is estimated to have existed nearly as long as humans. Historically, TB infection has been known by different names: white plague, phthisis, and consumption. Most people who are exposed to TB generally recover, usually without even knowing they were infected. Tuberculosis is spread as an aerosol and generally affects the lungs. When an infected person

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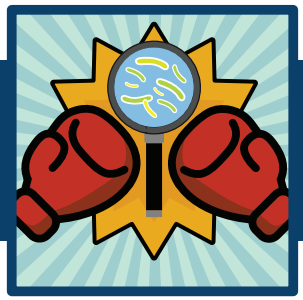

exhales, coughs, sneezes, or even sings, the TB bacteria is carried on droplets and stays suspended in the air for hours. When someone inhales these minute droplets, the TB is carried into the lungs where it attaches to cells within the lung.

There are two types of TB: active and inactive, also known as latent TB. People with active TB have multiple symptoms, including chest pain, weakness, chills, fever, and in later stages, coughing up blood. People with latent TB do not have symptoms, but unless they receive treatment, they can develop active TB later in life. Although TB is primarily considered an infection of the lungs, it can spread to lymph nodes, part of the lymphatic system, and can spread to other areas of the body, such as the brain, kidneys, or spine.

Latent TB: If a person has latent TB, the TB bacterium can live in different parts of the body for years without causing illness. An individual with latent TB does not have any symptoms and cannot spread the disease. However, because the individual does not have symptoms, the infection can go undetected. The only way an individual with latent TB can be diagnosed is if they have a positive TB test, using either a skin test or blood test. Unless an individual is traveling to a country which requires a negative TB test or has a job which requires such testing, latent TB can hide in different parts of the body for years. When the TB travels to other parts of the body, the immune system keeps working to rid the body of the invader. As in the lungs, granulomas can form around the TB in other areas of the body, not destroying the TB but surrounding it to limit the bacteria's ability to spread. However, as with granulomas in the lungs, the TB bacteria can reactivate at any time.

Antibiotics: Antibiotics are effective at treating bacterial infections, like TB. However, since their discovery, antibiotics have been misused. The prefix "anti-" means against. The afix "-bio" means life. Antibiotics are effective against living pathogens, like TB. They are not effective against non-living pathogens, like viruses.

Prior to antibiotics, treatments for infections included stressing good personal hygiene, like hand washing, and application of alcohol to treat infected surface wounds. For internal infections, treatments were more bazaar, like bloodletting where "infected" blood was drained from the patient. The discovery of antibiotics provided medical professionals with a valuable tool to treat both surface and internal bacterial infections. Antibiotics were seen as a true medical miracle.

Drug Resistance: Antibiotics fight bacteria in different ways by targeting specific parts of the bacteria's structure or internal function. With the discovery of antibiotics, the medical community had an effective treatment against bacterial infections. One unexpected benefit of the use of antibiotics was an eight-year increase in life expectancy. Despite the miracle of antibiotics, there has been misuse of antibiotics, including overprescribing and patients not taking antibiotics as prescribed. When patients take antibiotics, they can start to feel better in a few days. Thinking they are cured they stop taking the full prescription. However, not taking the full prescription means some bacteria may still be in the body. Having been exposed to the antibiotic but not killed, these bacteria develop resistance to future antibiotic treatments.

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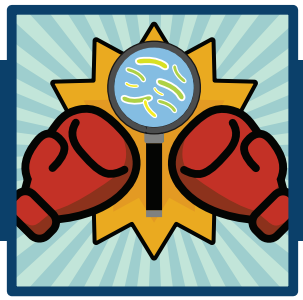

Drug resistant bacteria result from various processes, including but not limited to:

- **Natural Selection:** when initially exposed to an antibiotic most bacteria quickly die, but those bacteria who survive will pass on their resistance to subsequent generations.
- **Mutations:** random mutations in bacterial DNA can create a defense mechanism against antibiotics by preventing the antibiotic from reaching its targeted structure or produce enzymes which neutralize the effects of an antibiotic.
- **Rapid Reproduction:** bacteria reproduce rapidly, sometimes within minutes. If bacteria are resistant to antibiotics, the ability to reproduce quickly increases the number of antibiotic-resistant bacteria.

Using data from their past research, Texas Biomed scientists had evidence which showed the importance of the immune system in fighting TB. The challenge they faced was finding effective ways to fortify the immune system to fight TB without harmful side effects caused by current antibiotic therapeutics. These scientists examined outcomes of research for other "invasive" conditions in their search for protocols that effectively strengthen the immune system. One such research paper focused on cancer and the effect of Host-Directed Therapies (HDTs) on the immune system. Data from cancer research indicate the addition of HDTs, like Vitamin D, boosts the immune system. The HDTs increase the effectiveness of conventional cancer treatments and shorten the duration of treatments. The Texas Biomed scientists translated the result from cancer research and developed in vitro experiments to test the effectiveness of HDTs on the immune system to improve TB treatments.

Host-Directed Therapies & Cell Death Pathways

All cells have a limited life span. At the end of the cell's lifespan, it experiences a cell death pathway. Although there are several cell death pathways, the two primary pathways associated with TB research are apoptosis (A-pop-**toe**-sis) and necrosis (neh-**CROW**-sis). Apoptosis is the "normal" cell death pathway. During apoptosis, the cell membrane stays in tack, keeping all contents inside as the body eliminates the cell.

However, when a cell is infected with TB, the bacteria activate specialized receptors embedded in the cell membrane. When activated, these specialized receptors shut off apoptosis and activate the necrosis cell death pathway. With the necrosis pathway activated, the cell membrane will rupture, releasing bacteria into the body for more TB to infect other cells. As infected cells undergo necrosis chemical signals alert the immune system to send macrophages (**MAC**-row-fayj), the first line of defense. Using affixes, the word macrophage is derived from Greek meaning "big eater". The prefix "macro" meaning big and the suffix "phage" meaning to eat. The word is correctly pronounced in several ways, such as **MAC**-row-fahj or **MAC**-row-fayj.

The macrophages surround cells infected with TB through phagocytosis (fay-go-sigh-**TOE**-sis). Once the cell is surrounded the macrophage destroys the cell and kills the TB bacteria. The macrophage then undergoes necrosis. As it breaks apart, the "dead" TB is released into the body which alerts other immune cells of the invader. These other immune cells migrate to the area to kill the TB. However, if there are too many TB bacteria, the other immune cells surround the infected cells. This forms a ball called a granuloma (gran-you-**LOW**-ma). Tuberculosis bacteria inside the granuloma are still alive but encapsulated and unable to infect more cells.

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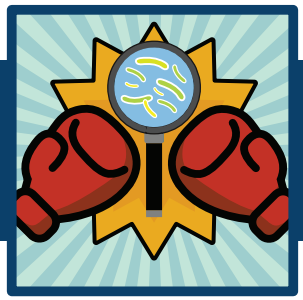

Antibiotics and TB

Antibiotics have been used successfully to treat TB infections, specifically rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB). Of these, RIF and INH are most frequently used to treat TB. But these antibiotics have harsh side effects such as chronic fatigue, blurred vision, easy bruising or bleeding. In addition, long-term use of antibiotics can cause tingling, numbness and burning in the hands and feet. Some of these side effects may be permanent.

The formation of granulomas by macrophages and other immune cells (macrophages, T cells, neutrophils, eosinophils, fibroblasts, collogen) present another problem for treatment. Antibiotics have limited success penetrating granulomas to reach the TB bacteria inside.

TB and Apoptosis

Boosting the immune system interferes with TB's ability to shut off apoptosis, meaning cells infected with TB do not break apart preventing TB from spreading. Although dead, the cell stays intact, and the body eliminates the cell, and the TB contained inside. It had been suspected that HDTs could be a mechanism to control TB's ability to shut off apoptosis, but until this study, there was limited research to support this idea. However, the positive results shown by the introduction of HDTs to treat cancer provided the necessary evidence to investigate HDTs effect on TB treatment.

Based on prior research and with information about HDTs from cancer research, Texas Biomed scientists sought to discover the impact of HDTs on TB. Unlike antibiotics which attack the TB bacteria, HDTs boost the immune system by increasing antimicrobial activities of immune cells to destroy TB while still inside infected cells. To treat granulomas the addition of HDTs changes the solubility of granulomas. By increasing the solubility of the surface of granulomas, it increases the ability of antibiotics to enter the granuloma and kill TB bacteria inside the structure.

Methods

Using invitro methods, this study sought to identify possible HDTs which could control the TB bacteria's ability to shut off apoptosis. The researchers at Texas Biomed used outcomes from previous research indicating specific anti-apoptotic proteins, MCL-2 (myeloid cell leukemia-1) and BCL-2 (B-cell lymphoma protein 2), embedded in cell membranes was activated by TB to over-ride or shut off the natural apoptotic cell death pathway. With apoptosis shut off, the necrotic cell death pathway is activated. Prior to this study, no one had investigated inducing apoptosis with HDTs to reduce TB infection.

Using blood samples from adults who tested negative for TB and bone marrow samples from mice, researchers isolated human and murine (rodent) macrophages. These samples were cultured using in vitro methods (conducted in a petri dish). Samples were exposed to a drug-resistant strain of TB. Over time, granulomas containing live TB formed. Granulomas were then separated for the experiment. Some granulomas were not

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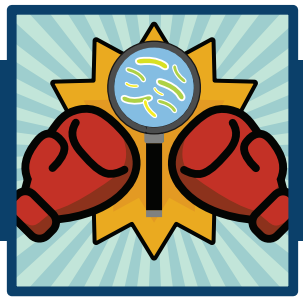

treated and served as a control. Other granulomas were treated with antibiotics currently used to treat TB, other granulomas were treated with combinations of HDTs, and others treated with HDTs and antibiotics.

A total of 10 experiments were conducted, each conducted over a four-day period. Applying the scientific method, the first experiment was a control, meaning no interventions or treatments were applied to the cultured human and murine granulomas. Data from this experiment provided a baseline to which the scientists could compare effectiveness of antibiotics alone and combined treatments on macrophages and granulomas infected with TB.

Results

In total, 10 experiments were conducted, including the control. To determine the effectiveness of various doses of HDTs on murine macrophages, the researchers counted and compared the number of Culture Forming Units (CFUs). Applying in vitro techniques, the researchers used petri dishes with an agar nutrient gel, called a substrate. When TB bacteria is placed on the nutrient substrate, it will form clusters of CFUs.

Murine Macrophages: In this stage of the experiment, scientists exposed murine macrophages to various doses of HDTs to evaluate the effectiveness of HDTs on macrophages by examining impact on cell death pathways. The number of CFUs are indicators of how different doses affect apoptosis and necrosis. If HDTs are not successful, the TB bacteria will cause necrosis. The cell membranes will rupture, releasing TB into the substrate, forming large numbers of CFUs. But if HDT treatment is successful, apoptosis will not be disrupted, meaning cell membranes will stay intact and the TB bacteria will be safely contained. This will reduce the number of CFUs.

The data provides evidence about how the addition of the HDTs at various doses affects cell death pathways and impacts the spread of TB. Overall, the addition of HDTs reduced the amount of TB CFUs. However, data indicate combinations of HDTs were more effective at reducing TB CFUs indicating a reduction in the activation of necrosis (Fig. 3). After a four-day period, macrophages were lysed (lysed), meaning broken open to evaluate how quickly HDTs could reduce the number of TB bacteria. The number of CFUs were counted and compared (Fig. 4).

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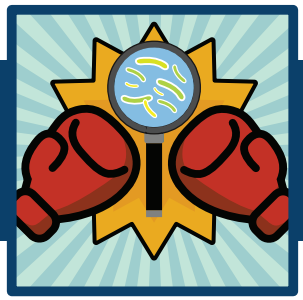
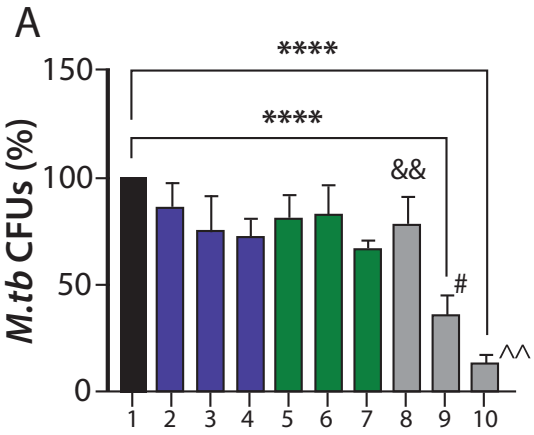


Figure 3. MDMs infected with TB then treated with HDTs (see Legend).



LEGEND

1. Control
2. 0.1 μM S
3. 1 μM S
4. 10 μM S
5. 0.1 μM ABT
6. 1 μM ABT
7. 10 μM ABT
8. 1 μM S + 1 μM ABT
9. 10 μM S + 1 μM ABT
10. 10 μM S + 10 μM ABT

NOTE: Colony Forming Units (CFUs)

Figure 4. CFU Comparison

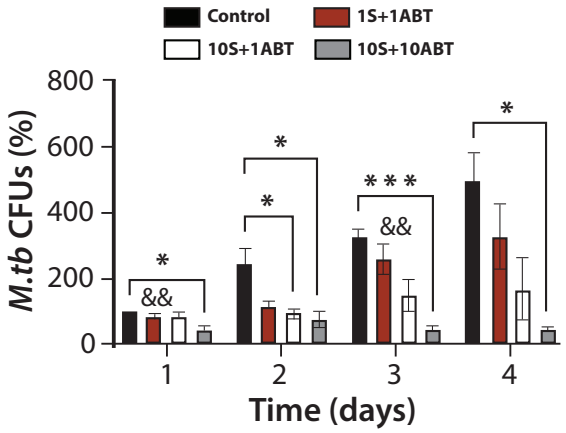
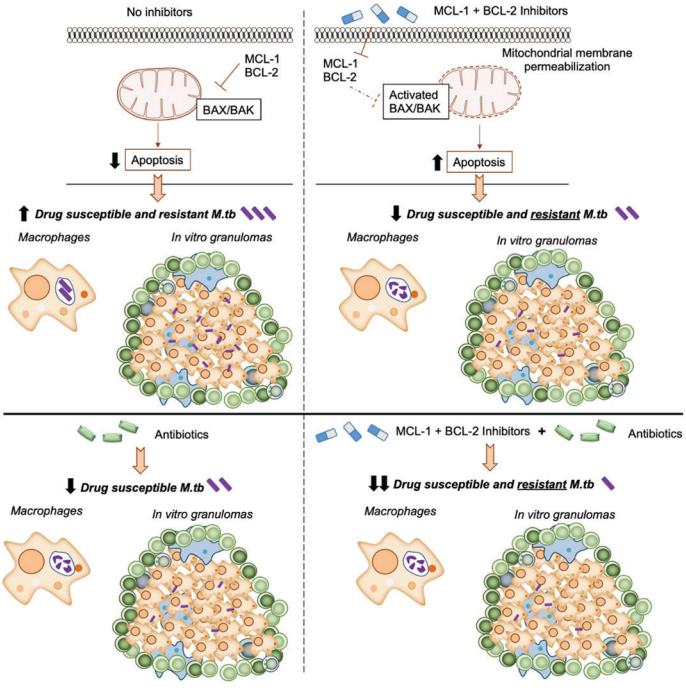


Figure 5. Comparison Between Treatment With and Without HDTs



Source: *ScienceDirect© (https://doi.org/10.1016/j.biopha.2023.115738)*

Human Granulomas and Macrophages: Developing effective treatments for granulomas provides a greater challenge. Granulomas are formed by macrophages and other immune cells that encapsulate live TB bacteria. Granulomas isolate TB bacteria, but the cells which contain the TB also create a barrier for antibiotics. Antibiotics have difficulty penetrating the granuloma structure which increases the duration of treatment with antibiotics. As with the macrophage data, treating granulomas with HDTs significantly reduces the number of live TB bacteria within granulomas (Fig. 5).

Conclusion

Applying information from cancer research, Texas Biomed TB researchers used in vitro protocols to test and compare the effectiveness of HDTs as a potential addition to antibiotic treatment. Their findings indicate HDTs reduce cell necrosis and reduce the spread of TB, including drug resistant TB. After four days, the scientists compared the number of CFUs in untreated (control) and treated (intervention) petri dishes. The data revealed a significant difference in the number of CFUs formed by each of the two groups. Macrophages treated with HDTs had a significantly lower number of CFUs

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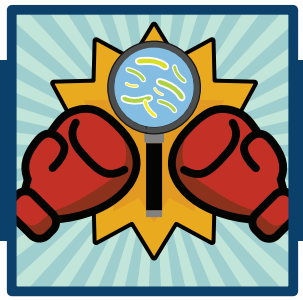

than untreated macrophages. The lower number of CFUs indicate the addition of HDTs reduces the occurrence of necrosis cell death pathways in murine macrophages infected with TB.

Data from in vitro experiments on human granulomas and human macrophages show similar results as fewer CFUs were associated with human granulomas and human macrophages treated with HDTs. With granulomas, it is assumed HDTs make the wall of the granuloma more soluble which increases antibiotics ability to enter the granuloma and attack the TB bacteria.

Limitations

As with all experiments, scientists evaluate the research methods to identify limitations which raise questions which require future research. There are several limitations in this study. These data are based on a four-day in vitro protocol. Would a longer in vitro study reveal different results? Another limitation is the short-term viability of human granulomas during in vitro studies. It is known that in in vitro settings, human granulomas only last for 12 days, whereas in the human body, granulomas can last for months or years. Although data indicate HDTs may be effective to add to antibiotic treatment for TB, this study was conducted in controlled situation within a lab.

Recent TB research indicates other immune cells embedded in the surface of the granuloma may deteriorate over time, releasing live TB bacteria into the body. This raises additional research questions: 1) What is the lifespan range of granulomas in vivo (in living organisms) and 2) When is the optimal time to introduce HDTs for maximum impact. As this is an innovative approach to treating TB with HDTs, human clinical trials require additional data. Although the findings of this study are promising, a wider base of data is needed.

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