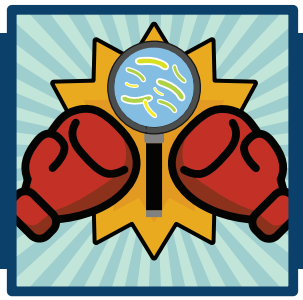
UNIT: TUBERCULOSIS

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

Activity 3C: *Consensus Mapping*



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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 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.

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 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.

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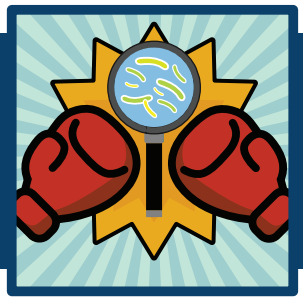


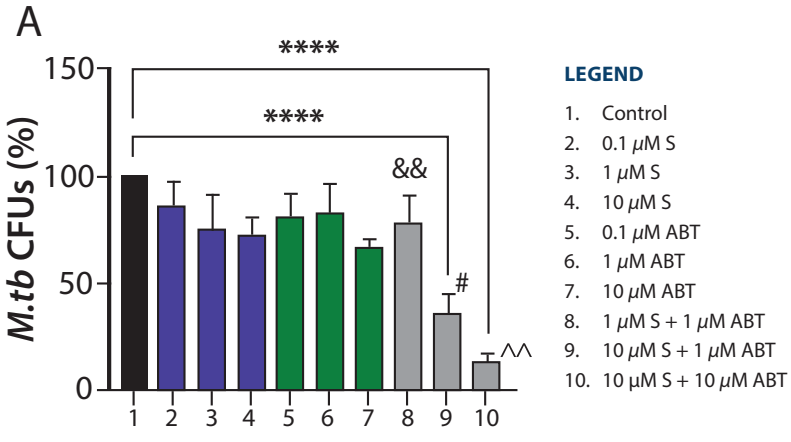
Results

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.

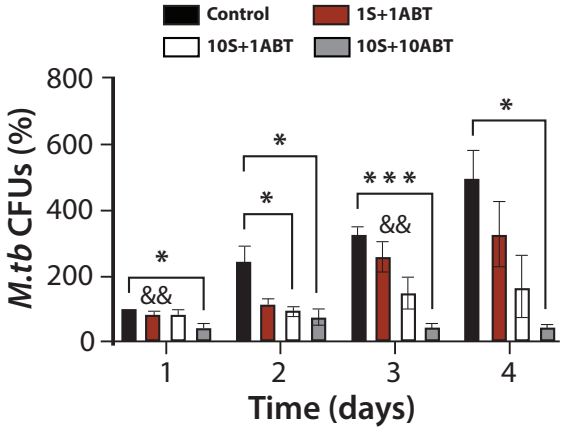
The data provides evidence about how the addition of the HDTs at various doses affects cell death pathways and impacts the spread of TB. Overall, the addition of HDTs reduced the amount of TB CFUs. However, data indicate combinations of HDTs were more effective at reducing TB CFUs indicating a reduction 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).

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



NOTE: Colony Forming Units (CFUs)

Figure 4. CFU Comparison



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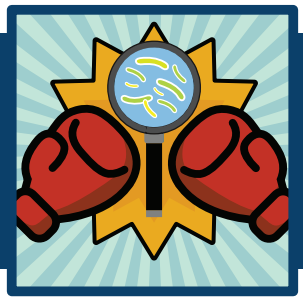
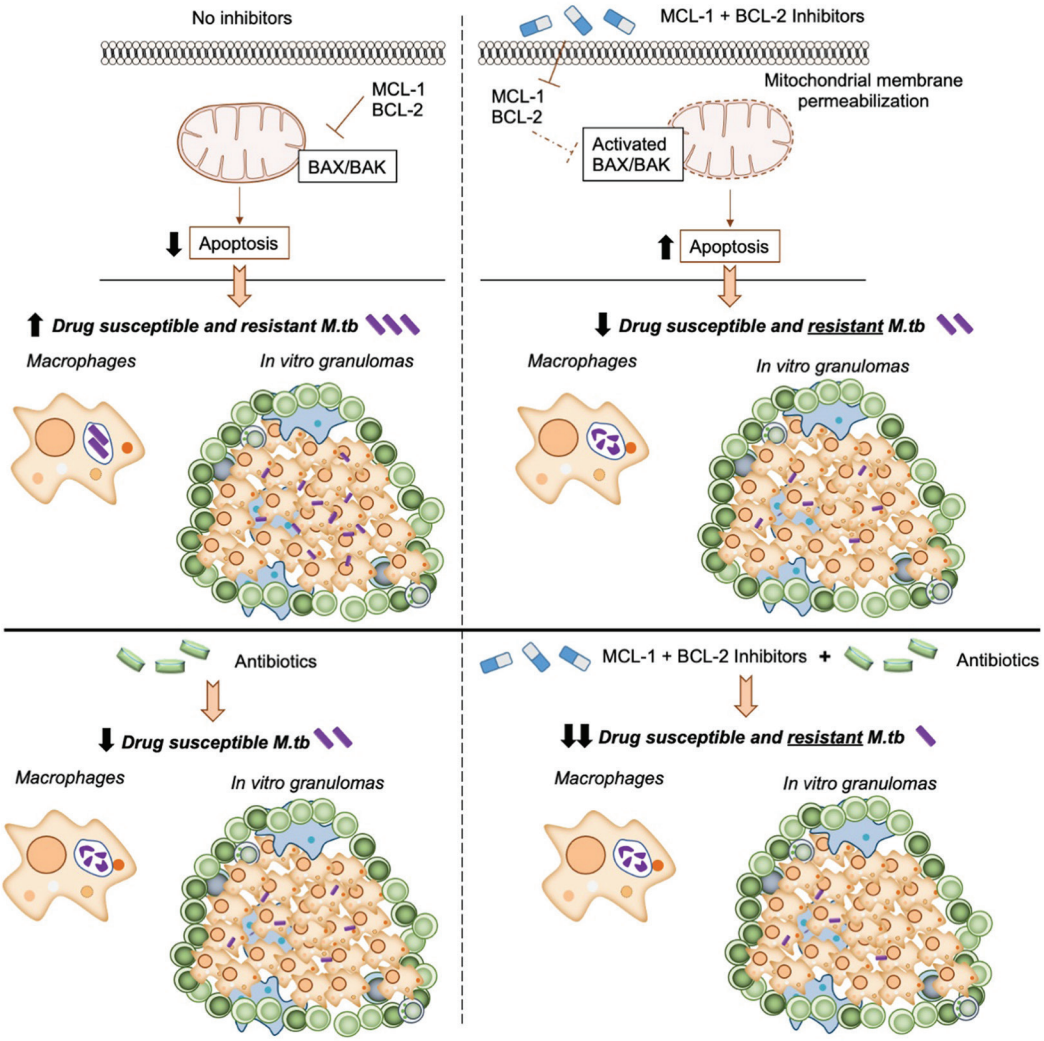


Figure 5. Comparison Between Treatment With and Without HDTs



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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).