GOOD NEWS!
There's a TB Killer on the Loose!

Photo Credit: CDC Site: phil.cdc.gov

TEXAS BIOMEDICAL RESEARCH INSTITUTE
HEALTH STARTS WITH SCIENCE

Transformation of Original Research Article

Cyktor, J.C., Carruthers, B., Stromberg, P., Flano, E., Pircher, H., & Turner, J. (2013). Killer cell lectin-like receptor G1 deficiency significantly enhances survival after Mycobacterium tuberculosis infection. Journals ASM.org, 81(4), 1090-1099.

Abstract:

Mycobacterium tuberculosis, referred to as ***M. tuberculosis (too-burr-cue-LO-sis)***, is a bacterial infection primarily found in the lungs. When the bacterium, commonly known as TB, invades the lungs, the body's immune system is triggered, causing the ***thymus (THIGH-mus)*** gland to produce specialized immune system cells, called T cells. The surface of T cells are covered with receptors, referred to as T Cell Receptors (TCR). These receptors allow T cells to recognize specific molecules called ***antigens (AN-tuh-jens)***. An antigen is a produced by a foreign substance, such as a bacteria, virus or even cancer cells. The presence of antigens activates the immune system. However, some receptors on the T cell do not recognize antigens. Once such receptor is known as the ***killer cell lectin-like receptor G1 (KLRG1)***. Instead of recognizing antigents, the KLRG1 receptor "sees" different receptors on other cells.

To develop effective treatments and even a cure for TB, researchers need to better understand how the presence or absence of different T cell receptors impact TB infection survival rates. For the study, mice were infected with TB. The researchers focused on KLRG1 receptors being expressed on specific subtype of T cell known as CD4 T cells. The data show mice infected with TB whose T cells lacked KLRG1 ($KLRG1^{-/-}$) lived longer with significantly lower levels of TB infection. In other words, when the KLRG1 receptor is absent, T cells are better able to trigger an immune response to TB infection. Outcomes of this study indicate an inverse or indirect relationship between KLRG1 and TB survival rates.

Background:

The tuberculosis bacterium is a contagious ***pathogen (PATH-oh-jen)*** which can be deadly. Since the discovery of the antibiotic ***streptomycin (strep-tow-MY-cin)*** in 1943, researchers have developed more effective treatment. However, TB continues to be a global health issue, even in the US.

Tuberculosis enters the body through the lungs. The thymus gland, located at the base of the throat, is a critical component of the immune system. This endocrine gland produces T cells to fight infections, including TB. Because these specific immune cells are made in the thymus, they are called T cells. But not all T cells are the same. Embedded in the surface of T cells are protein receptors. These receptors have different shapes which fit specific proteins. The different structure of each receptor mean T cells attach to different proteins on the surface of TB bacteria, resulting in different immune responses from the T cell.

How a T cell looks is called the cell's ***phenotype (FEE-no-type)***. The difference in a T cell's phenotype is determined by what is or is not on the surface of the T cell. All T cells have receptors embedded in the cell membrane, referred to as T cell receptors (TCR). Some T cells have killer cell ***lectin-like receptors G1 (KLRG1)*** on the surface. In this study, researchers focused on a specific subtype of T cells, called a CD4 T cell. Curiosity is a driving force behind science research. In this case, the researchers were curious if and how the presence of KLRG1 receptors affected the function of CD4 T cells to combat TB infection.

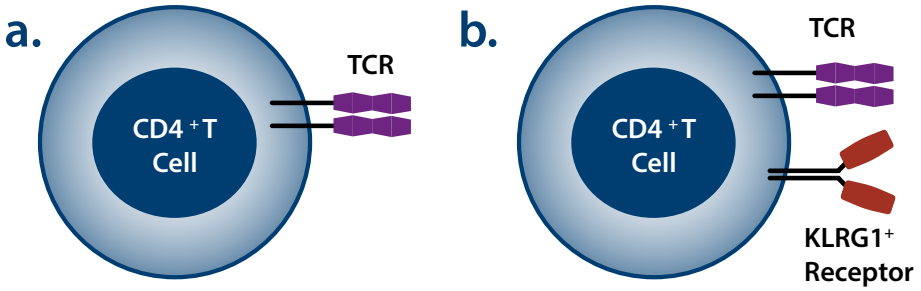

FIG 1 Cell phenotype. (a) $CD4^+$ T cell with T Cell Receptor (TCR), without KLRG1 receptor ($KLRG1^{-/-}$). (b) $CD4^+$ T cell with TCR and $KLRG1^+$ receptors.

NOTE: $^+$ indicates receptor is present and $^{-/-}$ indicates receptor is absent.

There have been other studies done about the connections between KLRG1 on CD4 T cells; however, these previous studies were done ***in vitro***, meaning the study was not conducted on living organisms. Very simply, this means T cell functions were studied in a petri dish. In vitro studies are valuable and provide scientists with valuable information. But, how a cell behaves in a petri dish may be different than how it behaves in a living organism. In this study, the researchers conducted an ***in vivo*** experiment. An in vivo experiment is conducted with living organisms, in this case mice.

The researchers compared survival rates of two different groups of mice infected with TB. One group of mice had T cells with KLRG1 receptors (control group) while the other group of mice had T cells without KLRG1 receptors (experimental group). The study data indicate mice infected with TB whose T cells did not have KLRG1 receptors ($KLRG1^{-/-}$) had a significantly higher survival rate. The researchers identified a possible correlation or connection between $KLRG1^{-/-}$ and the effectiveness of $CD4^+$ T cells. T cells that are $KLRG1^{-/-}$ had increased numbers of CD4 T cells. The CD4 T cells secrete ***gamma interferon (gam-ma inter-FEAR-on)***. Data from the study provides evidence that when gamma interferon is present, the severity of TB infection in mice is reduced. In other words, CD4 T cells help mice live longer. The presence of KLRG1 receptors seems to reduce

the effectiveness of CD4T cells which decreases the release of gamma interferon. Understanding how different T cell receptors impact one another is important and will help scientists develop more effective treatments for TB infection in humans.

Method:

The study was conducted within a Biohazard Safety Lab 3 (BSL3). Labs are placed into four categories: BSL 1, BSL 2, BSL 3, and BSL 4. A BSL 1 lab is found in most middle and high schools where experiments are relatively low risk, requiring minimum personal protective equipment (PPE) such as aprons, safety goggles, and close-toed shoes. Within a BSL 2 lab, scientists investigate infectious diseases which can cause sickness, but for which there are proven treatments and vaccines. Within a BSL2 lab, gloves need to be added to the PPE protection. Diseases, such as influenza, are studied in BSL2 labs.

A BSL3 lab requires more PPE as the diseases studied are highly contagious and always have airborne transmission. However, there are effective treatments or vaccines available. BSL3 researchers wear head-to-toe PPE, including a disposable full-body protective suit, double layers of gloves, face shields, booties, and a portable respirator. Because TB is highly contagious with some effective treatments, all TB studies are conducted within BSL3 labs. Finally, the BSL4 lab is reserved for contagious diseases with no known treatment or cure. Research for infectious diseases, such as Ebola and hemorrhagic fever, are conducted in the BSL4 lab. Researchers wear maximum protective gear which includes multiple layers of gloves and a full-body positive-pressure suit. When wearing the suit, researchers need to be connected to an oxygen supply. It takes months or even years of training before a researcher is qualified to work in the BSL3 or BSL4 lab.

Two types of mice were infected with TB: mice which were genetically bred to not have the KLRG1 ($KLRG1^{-/-}$) and wild-type mice whose genetics were not altered. All mice were observed daily over a period of 600 days to compare how the two different types of mice responded to the TB infection. The study was conducted twice to ensure repeatability and reliability of data. Study one included 25 wild-type and 25 $KLRG1^{-/-}$ (n=25 wild-type; n=25 $KLRG1^{-/-}$). Study two included 30 wild-type mice and 30 $KLRG1^{-/-}$ mice (n=30 wild-type; n=30 $KLRG1^{-/-}$).

Before scientists are granted permission to conduct in vivo studies, there are numerous safe-guards in place. These include multiple review committees comprised of scientists and community members who evaluate the proposed research, including the methods which involve animals. Once past the institutional safe guards, the proposed study goes to the funding agency, such as the National Institutes of Health (NIH) which has additional rigorous review committees. At the NIH level, only 18% of proposed grants are approved. When an NIH grant is funded, there is one more review which needs to be done before the study can begin. The Institutional Animal Care and Use Committee (IACUC) reviews all research studies which involve animals, in this case mice. The IACUC evaluates the study to ensure the least number of animals necessary are included in the study and animal care standards are appropriate. Only after receiving approval at all levels will the study begin.

Data:

Mice were infected with TB and observed for 600 days comparing survival rates between the wild-type and $KLRG1^{-/-}$ groups. The outcomes from study 1 (n=25 wild-type; n=25 $KLRG1^{-/-}$) are shown in Figure 2. The first wild-type mouse succumbed to TB on day 334, whereas the first $KLRG1^{-/-}$ succumbed on day 516.

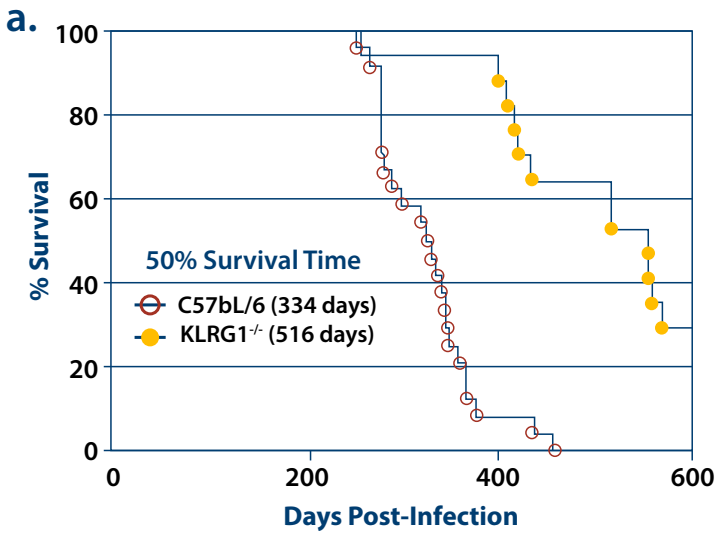

FIG 2 Survival of wild-type or KLRG$^{-/-}$ mice with ***M. tuberculosis*** burden.

As indicated on the graph, there is a significant difference in the survival rates of mice whose T cells did not have the KLRG1 receptor. When examining lung tissue, there was significant differences between the wild-type and KLRG1$^{-/-}$ groups. Specifically, the wild type showed faster disease progression. The level of inflammation and thickening of tissue, called granulomas (gran-u-LOW-mahs) was greater in wild-type mice than in that of the KLRG1$^{-/-}$ mice (Table 1).

TABLE: 1 Microscopic lung tissue from M. tuberculosis-infected mice

	Characterization	
Day	Wild-Type	KLRG1$^{-/-}$
90	Mild multifocal areas of unorganized granulomatous inflammation.	Mild multifocal areas of unorganized granulomatous inflammation.
120	Moderate to mild multifocal to coalescing granulomatous	Mild multifocal unorganized granulomatous foci
150	Coalescing areas of granulomatous inflammation	Moderate multifocal areas of unorganized granulomatous inflammation

Figure 3 compares the number of CD4^{+} T cells that express KLRG1 receptors at specific times after the mice were infected with TB bacterium.

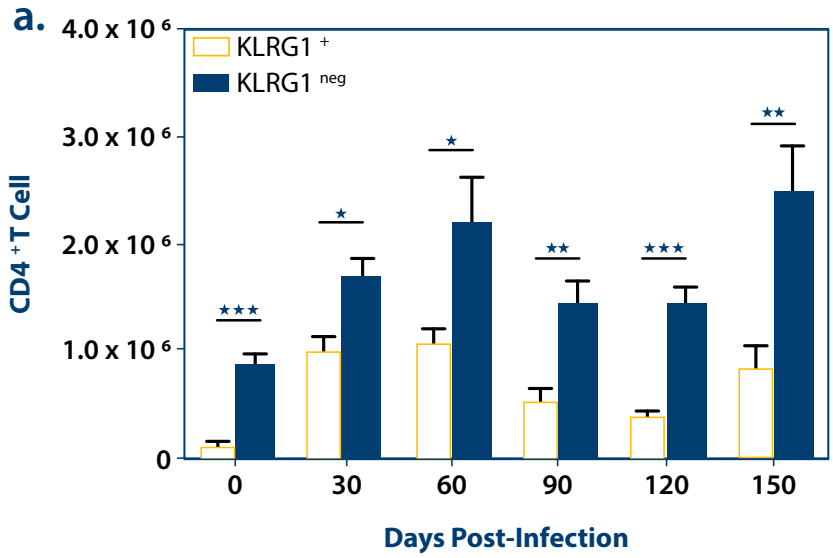

FIG 3 Pulmonary composition of KLRG1^{+} or KLRG$^{-/-}$ T cells post infection of M. tuberculosis.

NOTE: KLRG1+ are T cells obtained from wild-type mice and KLRG1neg are T cells from mice genetically lacking KLRG1 receptors.

Results:

Mice lacking KLRG1 receptors had more activated CD4^{+} T cells. It is known that CD4^{+} T cells secrete gamma interferon which provides a level of protection against ***M. tuberculosis*** infection. In other words, when KLRG1 receptors are absent, the levels of gamma interferon increased, indicating CD4^{+} T cells were more effective at protecting mice during a chronic TB infection when KLRG1 was absent.

Discussion:

When lungs are infected with ***M. tuberculosis***, the bacteria multiply causing inflammation which triggers the thymus to release T cells. The T cells have different receptors on the surface, each shaped to interact with a specific protein on an antigen. This initial ***in vivo*** study provides clear evidence that when KLRG1 receptors are absent, the survival rate during a chronic TB infection is significantly extended. This study suggests T cells with a KLRG1 phenotype have a significant impact on disease progression while T cells lacking KLRG1 receptors increase survival rates. However, these data show T cell responses naturally degrade over the course of an ***M. tuberculosis*** infection. These data also provide further evidence that KLRG1 receptors play a role in the degradation of T cells over time.

The researchers believe these findings are relevant to clinical treatment of TB. By ensuring optimal T cell response throughout the duration of a TB infection, the duration of infection could be significantly reduced. KLRG1 is called an exhaustion marker as it is expressed on T cells that are "tired", meaning the T cell has already worked too hard or is old (yes, all body cells age). When KLRG1 is expressed, it tells the T cell not to respond. When KLRG1 is removed, the 'off' signal is gone and the T cell can respond.