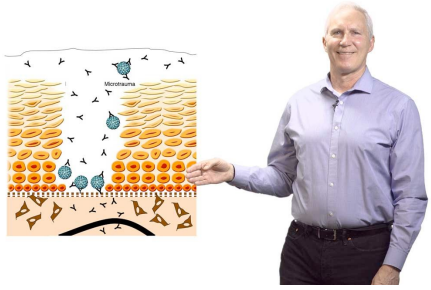
Session 10: The Immune Response in Health and Disease

Overview:
This session evaluates two examples of the interaction between pathogens and the human immune system. First, it characterizes human papillomavirus (HPV) pathogenesis and discusses how the molecular mechanisms of viral entry allow the HPV vaccine to work with high efficacy. Second, this session provides an overview of tuberculosis pathogenesis, the life cycle of the *M. tuberculosis* bacteria, and how *M. tuberculosis* uses the host immune system to increase infectivity.

First video:
Title: Human Papillomavirus (HPV) Vaccines to Prevent Cancer: Why Do HPV Virus-Like Particle Vaccines Work So Well?
Speaker: John Schiller
Time: 34:26

Concepts: Human Papillomavirus (HPV), HPV susceptibility to neutralizing antibodies, B cell receptor/Virus-like particle complex, and importance of viral infection timeline



Questions for First Video:

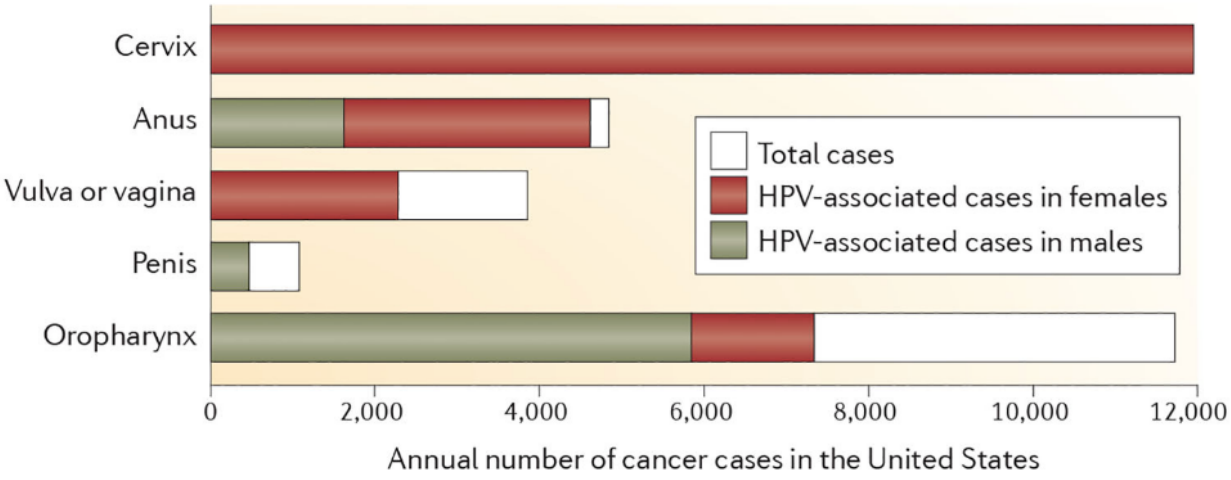
1. State whether the following statements are true or false. If false, explain why.
    a. The main part of the immune system activated by vaccines to prevent disease is the B cell.
    b. After vaccination, the immune system will develop a large amount of plasma cells. These cells will remain high for the rest of the life of the individual and will help protect the individual from future infection.
    c. The HPV vaccine induces both IgA and IgG antibodies.
    d. Single-dose vaccination is appropriate for all subunit vaccines.
    e. Immunity to HPV can be passively transferred (i.e., via serum) between individuals.
    f. Pattern recognition is attributed to both innate and adaptive immunity.
    g. HPV virions prefer to be released from cells of the basal epithelial layer so that they can immediately infect more cells.

2. Which of the following factors are important considerations when developing vaccines? (Select all that apply)
    a. Strong response by the innate immune system.
    b. Increased number of memory B cells.
    c. Increased number of plasma cells.
    d. Increased number of Tregs.
    e. Increased number of cytotoxic T cells.

3. Mary is developing a vaccine against HIV. Which of the following are factors that Mary should consider?
    a. Infection mechanism of HIV.
    b. Choosing a target with high selective pressure.
    c. Choosing a target with low selective pressure.
    d. Mutability of the virus.
    e. None of the above.

4. Discussion Question: Human Papillomavirus is a sexually transmitted virus that could cause cancer.
    a. Briefly describe why the method of transmission of this virus has been a problem for the compliance in vaccination.

    b. Shown below are the rate of HPV-induced cancers compared to the total incidence of cancer.



Schiller JT and Lowy DR (2012) Nat Rev Microbiol

    i. In females, cervical HPV is the leading inducer of cervical cancer. Pap screening decreases the incidence of cervical cancer by 80%. How might this statistic affect the vaccination rate?

    ii. There is an expectation that the HPV vaccine will help reduce the incidence of HPV-induced cancer, especially within low-income populations. Why?

5. The HPV vaccine uses viral-like particles (VLP) to induce antibody production in patients. Recent research suggests that B cells are capable of “antigen-specific pattern recognition.”
    a. Explain how spatial organization of the BCR relates to the strength of a B cell response. Be sure to include the molecular interactions involved.

    b. How does B cell pattern recognition relate to efficacy of the HPV virus-like particle vaccine? Compare and contrast the BCR response to VLPs with that of non-viral-like particles.

    c. How could HPV evolve resistance to the HPV vaccine?

    d. What would be the consequence for vaccine-mediated immunity if HPV gained faster entry into epithelial cells (seconds rather than hours) through mutation? Briefly explain.

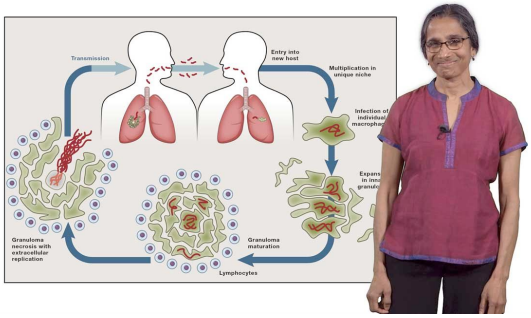
6. Why, in general, is the immune response to a viral infection stronger than the immune response typically induced by a subunit vaccine?

7. The HPV subunit vaccine is much more effective than other subunit vaccines, such as HBV. By comparing the HPV and HBV virus-like particle (VLP) vaccines, researchers were able to hypothesize what aspects of a VLP lead to increased efficacy. Name two of these aspects.

8. Explain how the infection cycle of HPV makes it particularly susceptible to neutralizing antibodies.

Second video:
Title: An Introduction to Tuberculosis: The Pathogenic Personality of the Tubercle Bacillus
Speaker: Lalita Ramakrishnan
Time: 34:44

Concepts: Overview of tuberculosis (TB), TB prevalence worldwide, life cycle of TB, TB pathogenesis (infection of macrophages & granulomas), efflux pumps, and TB vaccine



Questions for Second Video:

1. Which of the following factors contribute to tuberculosis prevalence? (Select all that apply)
    a. Hygiene.
    b. Access to available treatment.
    c. Treatment compliance.
    d. HIV prevalence.
    e. None of the above.

2. State whether the following statements are true or false. If false, explain why.
    a. The tuberculosis (TB) pathogen is a commensal bacterium.
    b. All macrophages in the lung have the same effector functions.
    c. The TB pathogen co opted genes that were essential for life in soil to adapt to life in a vertebrate host.
    d. Individuals can have pre-existing immunity to TB.

3. Drug-resistant TB is a growing problem. How might increased treatment compliance impact the development of drug resistant TB in a population? Briefly explain.

4. TB lacks many of the “classical” factors found in other pathogens.
    a. What are the evolutionary advantages and disadvantages of not sharing some of the factors found in other pathogens (e.g. capsule, flagella and pili)?

b. How does TB overcome the lack of these factors to be successful?

5. The efflux pump is important for TB infectivity.
    a. Why did TB evolve to have efflux pumps? Briefly explain.

    b. Describe a mechanism by which efflux pumps are beneficial for TB pathogenesis.

    c. The TB vaccine uses live-attenuated bacteria. What are some complications of the use of live-attenuated bacteria in vaccines? Briefly explain.

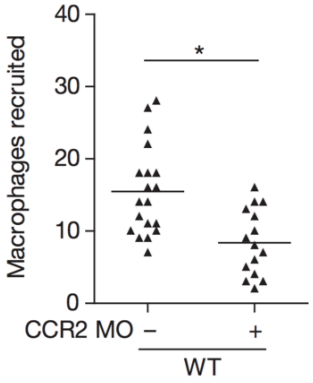
6. The tuberculosis (TB) pathogen can infect many types of tissues. However, TB is considered a “dead-end” disease if it infects tissues other than the lung. Explain why this is the case.

7. Why might chronic smoke inhalation be a risk factor for TB?

8. Even though TB *can* be treated with antibiotics, explain the clinical limitations that limit the efficacy of antibiotic treatments.

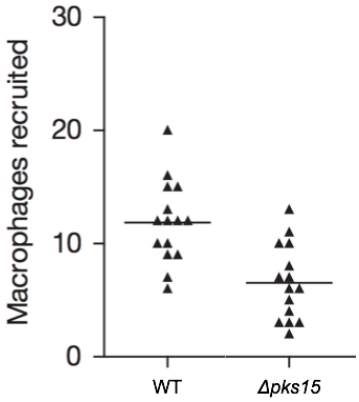
9. Are interactions of the TB bacterium with commensal bacteria in the airway helpful or harmful to its ability to infect? Briefly explain.

10. A group of researchers sought to identify disease-promoting molecular interactions between the tuberculosis (TB) bacterium and the host immune system. They hypothesized that the chemokine receptor CCR2 on permissive macrophages was necessary for their recruitment to sites of infection. To test this hypothesis, they knocked down CCR2 expression using morpholino (MO) technology (similar to siRNA) in zebrafish, then infected the fish with the TB pathogen and quantified macrophage recruitment to the site of infection. (Reference: Cambier, Nature 2014)

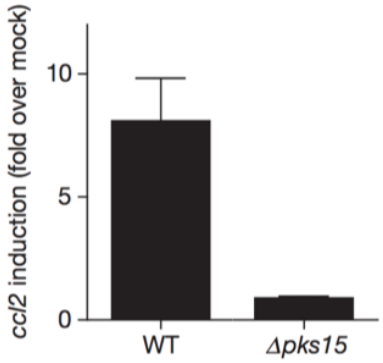


a. Based on the data above, what can you conclude about the role of CCR2 in permissive macrophage recruitment to sites of TB infection?

b. Next, the researchers sought to identify surface components of the TB pathogen that are necessary for macrophage recruitment. They hypothesized that a lipid known as PGL may play a role. Bacteria deficient in the gene *pks15* lack PGL at their surface. Zebrafish were injected with bacteria deficient in *pks15*, and recruitment of macrophages to the site of infection was measured. Based on the data below, what can you conclude about the role of PGL in the interaction between host and microbe?



c. Finally, the researchers sought to determine a mechanism for the role of bacterial surface components in permissive macrophage recruitment. They measured mRNA expression of the chemokine CCL2 in the tissues around injected wild-type and *pks15*-deficient TB bacteria. Based on the data below, develop a hypothesis to explain the interactions of CCR2, PGL, and CCL2 as they relate to the host response to TB infection.



d. Do the following data support or refute your hypothesis? Briefly explain. Note: CCR2-morphant (MO) fish are treated with CCR2 morpholino.

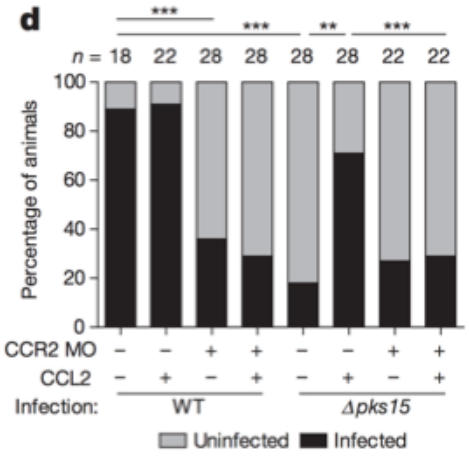
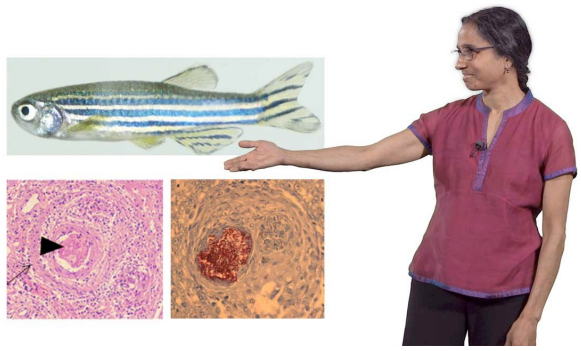


Figure legend: Wild-type and CCR2-morphant (MO) fish, with or without the addition of CCL2, were infected with wild-type or *Δpks15* TB bacterium. Graph shows the percentage of fish that were infected (black) or uninfected (grey) after 5 days.

Optional video:
Title: The Troublesome Tubercle in Tuberculosis
Speaker: Lalita Ramakrishnan
Time: 29:42

Concepts: TB use of granuloma to attract macrophages and increase infectivity, *Mycobacterium marina* - a model to study TB in zebrafish, granuloma promotes growth of the TB bacteria, and expansion of disease via macrophage apoptosis



Questions for Optional Video:

1. State whether the following statements are true or false. If false, explain why.
    a. Granulomas are specific to TB infection.
    b. *M. Tuberculosis* co-opts host immune pathways for its own benefit.

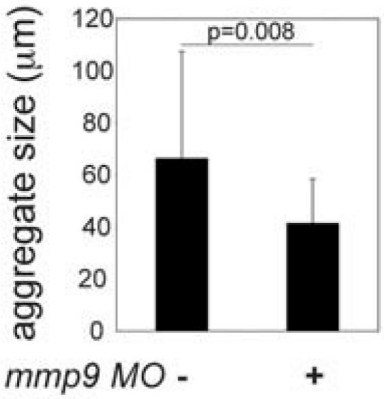
2. Briefly describe how the following immunological components aid tuberculosis pathogenesis.
    a. Macrophages

    b. Granuloma

3. Briefly describe two benefits of using *Mycobacterium marinum* as a model pathogen to study TB.

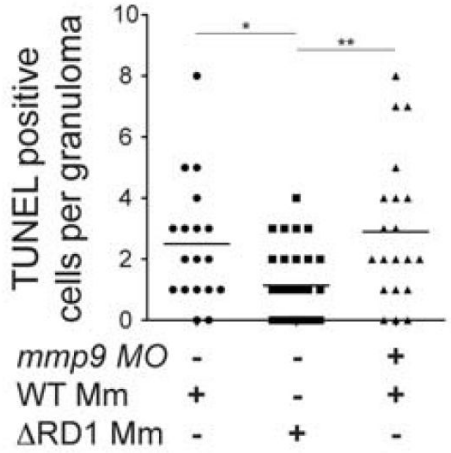
4. Compare and contrast foreign body and epithelioid granulomas.

5. Researchers found that matrix-metalloproteinase 9 (MMP9) secretion is induced in surrounding epithelial cells during TB infection. They sought to determine the role of MMP9 in TB infection. To do so, they knocked down MMP9 expression using morpholino (MO) technology (similar to siRNA) in zebrafish, then infected the fish with the TB pathogen and quantified granuloma size. (Reference: Volkman, Science 2010)



a. Based on the results above, how does MMP9 expression affect granuloma formation?

b. It is known that the tuberculosis (TB) bacterium expands its infection range by inducing macrophage apoptosis. The researchers sought to determine whether MMP9 plays a role in inducing macrophage apoptosis. To test their hypothesis, they measured rates of TUNEL positive cells (TUNEL is a marker of damaged DNA from cell death) in zebrafish granulomas induced by TB bacterium infection. In the figure below, mmp9 MO = knockdown of MMP9 by morpholino; WT Mm = infection with wild-type TB bacterium; ΔRD1 Mm = infection with TB bacterium lacking RD1, a genetic locus important for virulence of TB bacterium.



i. Is MMP9 involved in macrophage apoptosis? Briefly explain

ii. Is RD1 involved in macrophage apoptosis? Briefly explain.

iii. Design an experiment to identify other potential roles for MMP9 in TB pathology.

Answers for Session 10:

Questions for First Video:

1. State whether the following statements are true or false. If false, explain why.
    a. The main part of the immune system activated by vaccines to prevent disease is the B cell. **(True)**
    b. After vaccination, the immune system will develop a large amount of plasma cells. These cells will remain high for the rest of the life of the individual and will help protect the individual from future infection. **(False - it's vaccine dependent, but most vaccines will have a decrease of plasma cells over time)**
    c. The HPV vaccine induces both IgA and IgG antibodies. **(False - it only induces IgG antibodies)**
    d. Single-dose vaccination is appropriate for all subunit vaccines. (**False - not all subunit vaccines produce equally strong immune responses. Some (such as HBV) need multiple doses for efficacy.)**
    e. Immunity to HPV can be passively transferred (i.e., via serum) between individuals. (**True**)
    f. Pattern recognition is attributed to both innate and adaptive immunity. (**True**)
    g. HPV virions prefer to be released from cells of the basal epithelial layer so that they can immediately infect more cells. (**False - they prefer to be released from cells at the top of the epithelium, so that they are less likely to interact with immune components)**

2. Which of the following factors are important considerations when developing vaccines? (Select all that apply)
    a. Strong response by the innate immune system.
    b. Increased number of memory B cells.
    c. Increased number of plasma cells.
    d. Increased number of Tregs.
    e. Increased number of cytotoxic T cells.

3. Mary is developing a vaccine against HIV. Which of the following are factors that Mary should consider?
    a. Infection mechanism of HIV.
    b. Choosing a target with high selective pressure.
    c. Choosing a target with low selective pressure.
    d. Mutability of the virus.
    e. None of the above.

4. Discussion Question: Human Papillomavirus is a sexually transmitted virus that could cause cancer.
    a. Briefly describe why the method of transmission of this virus has been a problem for the compliance in vaccination.
    HPV is sexually transmitted. For religious/cultural reasons, populations haven't seen the importance of vaccination against HPV.

b. Shown below are the rate of HPV-induced cancers compared to the total incidence of cancer.
    i. In females, cervical HPV is the leading inducer of cervical cancer. Pap screening decreases the incidence of cervical cancer by 80%. How might this statistic affect the vaccination rate?
    Most cervical cancer cases can be prevented using pap screening. This could reduce vaccination rates as individuals wouldn't see the need for vaccination when the disease is preventable.

    ii. There is an expectation that the HPV vaccine will help reduce the incidence of HPV-induced cancer, especially within low-income populations. Why?
    Lower-income populations have less access to health care, including pap screenings. Therefore, the incidence in this population is higher and vaccination would have a bigger impact.

5. The HPV vaccine uses viral-like particles (VLP) to induce antibody production in patients. Recent research suggests that B cells are capable of "antigen-specific pattern recognition."
    a. Explain how spatial organization of the BCR relates to the strength of a B cell response. Be sure to include the molecular interactions involved.
    Even spacing and simultaneous engagement of individual BCRs by highly repetitive antigens leads to crosslinking of their associated tyrosine kinases. This amplifies the signal from the cell surface.

    b. How does B cell pattern recognition relate to efficacy of the HPV virus-like particle vaccine? Compare and contrast the BCR response to VLPs with that of non-viral-like particles.
    Oligomerization of the BCR by the HPV VLP vaccine leads to strong signals that induce long-lived plasma cells. In contrast, a BCR that binds to a monomeric component does not oligomerize with other BCRs, leading to a weaker signal and weaker immune response.

    c. How could HPV evolve resistance to the HPV vaccine?
    Mutations in the virus region mimicked by the VLP could arise, allowing the virus to evade host recognition (even if the host has been vaccinated).

    d. What would be the consequence for vaccine-mediated immunity if HPV gained faster entry into epithelial cells (seconds rather than hours) through mutation? Briefly explain.
    This could potentially reduce the effectiveness of the vaccine. The vaccine is effective because it takes advantage of the slow cellular entry of the virus. If the rate of entry increases, antibodies will have a lower chance of detecting the virus and mounting an immune response/ inhibiting viral entry.

6. Why, in general, is the immune response to a viral infection stronger than the immune response typically induced by a subunit vaccine?
    A real viral infection would provide many other signals to activate the immune system, such as TLR activation and induction of cytokines.

7. The HPV subunit vaccine is much more effective than other subunit vaccines, such as HBV. By comparing the HPV and HBV virus-like particle (VLP) vaccines, researchers were able to hypothesize what aspects of a VLP lead to increased efficacy. Name two of these aspects.
   Highly repetitive antigen display increases the strength of the B cell response; more rigid VLP may produce stronger response; overall size of particle may play a role; stability of VLP via disulfide bridges

8. Explain how the infection cycle of HPV makes it particularly susceptible to neutralizing antibodies.
   An HPV virion must interact with the basement membrane of the epidermis, which leads to cleavage of its capsid by extracellular proteases. After modification of the capsid proteins, the virion can then bind receptors on epithelial cells (keratinocytes) to begin infection. This whole process occurs over several hours, meaning that the virions are highly susceptible to antibody interference.

Questions for Second Video:

1. Which of the following factors contribute to tuberculosis prevalence? (Select all that apply)
   a. **Hygiene.**
   b. **Access to available treatment.**
   c. **Treatment compliance.**
   d. **HIV prevalence.**
   e. None of the above.

2. State whether the following statements are true or false. If false, explain why.
   a. The tuberculosis (TB) pathogen is a commensal bacterium. (**False - TB is an obligate parasite**)
   b. All macrophages in the lung have the same effector functions. (**False - there are microbicidal and permissive macrophages**)
   c. The TB pathogen co opted genes that were essential for life in soil to adapt to life in a vertebrate host. (**True**)
   d. Individuals can have pre-existing immunity to TB. (**True**)

3. Drug-resistant TB is a growing problem. How might increased treatment compliance impact the development of drug resistant TB in a population? Briefly explain.
   Increased treatment compliance will likely decrease the development of drug resistant TB. Lack of treatment compliance (i.e., not following the full course of treatment) allows the pathogen to evolve resistance to the treatment.

4. TB lacks many of the “classical” factors found in other pathogens.
   a. What are the evolutionary advantages and disadvantages of not sharing some of the factors found in other pathogens (e.g. capsule, flagella and pili)?
      Advantages: Harder to be recognized by the host immune system.

      Disadvantages: Not as infectious. The lack of some of these factors has an impact on the mobility and protection of the pathogen.

b. How does TB overcome the lack of these factors to be successful?
TB has a mechanism to coat its PAMPS (pathogen-associated molecular patterns) to avoid toll-like receptor (TLR) activation. In addition, TB releases factors to recruit a special type of macrophage that aids its infectivity. These mechanisms help TB to avoid the innate immune system, and fixes its motility problem.

5. The efflux pump is important for TB infectivity.
    a. Why did TB evolve to have efflux pumps? Briefly explain.
    TB evolved efflux pumps to survive the evolutionary battle against other bacteria in soil.

    b. Describe a mechanism by which efflux pumps are beneficial for TB pathogenesis.
    Efflux pumps decrease the efficacy of antibiotics/treatment as these pumps can be used by TB to expel antibiotics outside the cell.

    c. The TB vaccine uses live-attenuated bacteria. What are some complications of the use of live-attenuated bacteria in vaccines? Briefly explain.
    If the pathogen has high mutability, some live-attenuated bacteria could mutate back to a pathogenic state.

6. The tuberculosis (TB) pathogen can infect many types of tissues. However, TB is considered a "dead-end" disease if it infects tissues other than the lung. Explain why this is the case.
Lung infection is necessary for transmission of the pathogen (via coughing and sputum).

7. Why might chronic smoke inhalation be a risk factor for TB?
Chronic smoke inhalation likely leads to chronic inflammation in the lungs, which can damage the tissue and enhance the ability for a TB infection to take hold.

8. Even though TB *can* be treated with antibiotics, explain the clinical limitations that limit the efficacy of antibiotic treatments.
Patients need to be treated for 6 months with antibiotics; TB is prevalent in areas with limited access to healthcare/low socioeconomic status.

9. Are interactions of the TB bacterium with commensal bacteria in the airway helpful or harmful to its ability to infect? Briefly explain.
The interactions of TB with commensal bacteria are harmful, as the innate immune response that commensals activate can also clear TB.

10. A group of researchers sought to identify disease-promoting molecular interactions between the tuberculosis (TB) bacterium and the host immune system. They hypothesized that the chemokine receptor CCR2 on permissive macrophages was necessary for their recruitment to sites of infection. To test this hypothesis, they knocked down CCR2 expression using morpholino (MO) technology (similar to siRNA) in zebrafish, then infected the fish with the TB pathogen and quantified macrophage recruitment to the site of infection. (Reference: Cambier, Nature 2014)
    a. Based on the data above, what can you conclude about the role of CCR2 in permissive macrophage recruitment to sites of TB infection?
    CCR2 expression mediates recruitment of permissive macrophages to sites of TB infection.

b. Next, the researchers sought to identify surface components of the TB pathogen that are necessary for macrophage recruitment. They hypothesized that a lipid known as PGL may play a role. Bacteria deficient in the gene *pks15* lack PGL at their surface. Zebrafish were injected with bacteria deficient in *pks15*, and recruitment of macrophages to the site of infection was measured. Based on the data below, what can you conclude about the role of PGL in the interaction between host and microbe?
PGL mediates recruitment of permissive macrophages to sites of TB infection.

c. Finally, the researchers sought to determine a mechanism for the role of bacterial surface components in permissive macrophage recruitment. They measured mRNA expression of the chemokine CCL2 in the tissues around injected wild-type and *pks15*-deficient TB bacteria. Based on the data below, develop a hypothesis to explain the interactions of CCR2, PGL, and CCL2 as they relate to the host response to TB infection.
Host detection of PGL leads to release of CCL2, which recruits permissive macrophages to the site of infection via binding of CCL2 to macrophage CCR2.

d. Do the following data support or refute your hypothesis? Briefly explain. Note: CCR2-morphant (MO) fish are treated with CCR2 morpholino.
These data support the hypothesis that CCL2 is necessary for recruitment of permissive macrophages to the site of infection and helping infection to take hold, and support the hypothesis that host detection of PGL leads to CCL2 production (in the absence of exogenous CCL2).

Questions for Optional Video:

1. State whether the following statements are true or false. If false, explain why.
    a. Granulomas are specific to TB infection. (**False - granulomas are a widespread immune response**)
    b. *M. Tuberculosis* co-opts host immune pathways for its own benefit. (**True**)

2. Briefly describe how the following immunological components aid tuberculosis pathogenesis.
    a. Macrophages
    Tuberculosis infects permissive macrophages.

    b. Granuloma
    Macrophages induce epithelial cells to release a factor that recruits additional macrophages into the granuloma. This recruitment enhances infection of new macrophages and therefore spreads the disease.

3. Briefly describe two benefits of using *Mycobacterium marinum* as a model pathogen to study TB.
***M. marinum* infects fish the same way *M. tuberculosis* infects humans. This allows one to study the infectivity of this pathogen using zebrafish, an animal model that can be used to do genetic testing and is easy to handle in the laboratory. The traditional method to study TB in human cells is dangerous for researchers and requires special research centers. Because *M. marinum* doesn't infect humans to the same extent, it's less dangerous and can be handled with fewer precautions.**
***M. marinum* pathogenesis is similar, including the formation of granulomas.**

4. Compare and contrast foreign body and epithelioid granulomas.
   Foreign body granulomas attempt to wall off a foreign object (such as a thorn). They have low macrophage turnover and are non-inflammatory. Epithelioid granulomas are medically significant and typically surround a pathogen. They have high macrophage turnover and are inflammatory.

5. Researchers found that matrix-metalloproteinase 9 (MMP9) secretion is induced in surrounding epithelial cells during TB infection. They sought to determine the role of MMP9 in TB infection. To do so, they knocked down MMP9 expression using morpholino (MO) technology (similar to siRNA) in zebrafish, then infected the fish with the TB pathogen and quantified granuloma size. (Reference: Volkman, Science 2010)
   a. Based on the results above, how does MMP9 expression affect granuloma formation?
      MMP9 expression facilitates the growth of granulomas. When MMP9 is knocked down, granuloma aggregate size is smaller.

   b. It is known that the tuberculosis (TB) bacterium expands its infection range by inducing macrophage apoptosis. The researchers sought to determine whether MMP9 plays a role in inducing macrophage apoptosis. To test their hypothesis, they measured rates of TUNEL positive cells (TUNEL is a marker of damaged DNA from cell death) in zebrafish granulomas induced by TB bacterium infection. In the figure below, mmp9 MO = knockdown of MMP9 by morpholino; WT Mm = infection with wild-type TB bacterium; ΔRD1 Mm = infection with TB bacterium lacking RD1, a genetic locus important for virulence of TB bacterium.
      i. Is MMP9 involved in macrophage apoptosis? Briefly explain
         No - when MMP9 is knocked down, the number of TUNEL-positive cells is no different than the number in granulomas infected with wild-type TB bacteria.

      ii. Is RD1 involved in macrophage apoptosis? Briefly explain.
         Yes - RD1-deficient bacteria have reduced rates of apoptosis in their granulomas.

      iii. Design an experiment to identify other potential roles for MMP9 in TB pathology.
         Many possible answers: gene array to look for changes in gene expression in MMP9-deficient animals infected with TB; mass spec on tissues to look for protein/biochemical changes; pull-down assay to identify binding partners of MMP.