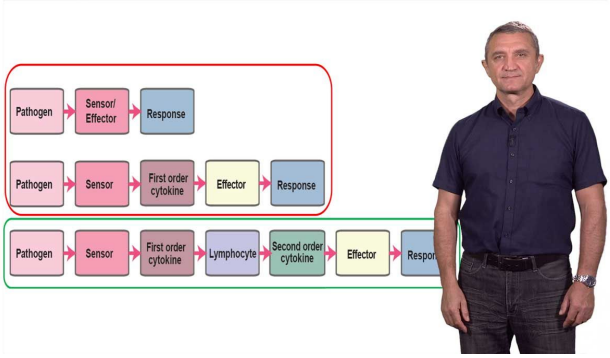
Session 2: The Inflammatory Response: Activation of the Innate Immune System

Overview:
Some of the classic symptoms associated with illness (inflammation, anorexia, fever, etc.) help the body fight disease and return to its normal state (homeostasis). This session will help you understand the molecular basis of inflammation. In summary, the body has specialized immune cells (sensor cells) that detect homeostasis disturbances and activate a series of responses that help clear the inflection and restore homeostasis. Finally, this session evaluates how some of these defenses, e.g. anorexia, can aid or disrupt recovery depending on the type of infection the body experiences.

First video:
Title: Introduction to Inflammation
Speaker: Ruslan Medzhitov
Time: 36:30

Concepts: Homeostasis, inflammation, sensors, effector cells, mediators, and resolution



Questions for First Video:

1. Nonspecific (innate) immune responses include
    a. inflammation.
    b. antigen-antibody complexes.
    c. immunoglobulin action.
    d. complement and memory T cells.
    e. memory B cells.

2. Cytokines
    a. are regulatory Toll-like receptors.
    b. prevent the inflammatory response.
    c. include interferons and interleukins.
    d. are immunoglobulins.
    e. include interleukins and complement proteins.

3. State whether the following statements are true or false. If false, explain why.
    a. Cytokines and Chemokines are effectors.
    b. A Neutrophil is a Sensor cell.
    c. Pathogens are required for activation of an inflammatory response.

4. Compare and contrast the following sets of terms. Provide examples.
    a. Homeostatic state, stress response (physiological inflammation), and inflammation.

    b. Structural feature recognition and functional feature recognition.

5. The inflammatory pathway includes inducers, sensors, mediators, and effectors.
    a. Defend why sensors and effectors have evolved to be cell-based entities, while mediators are cell-free entities?

    b. Imagine the opposite scenario and explain why it is less ideal.

6. Histamine is produced by basophils and mast cells found in nearby connective tissues as a response to foreign pathogens. Histamine helps to fight infection by increasing the permeability of the capillaries to white blood cells and other proteins, which facilitates their entry to the infected tissue.
    a. The following characteristic defines the role of the basophil, histamine, and white blood cell, respectively:
        i. Effector, Mediator, and Sensor.
        ii. Mediator, Mediator, and Sensor.
        iii. Sensor, Mediator, and Effector.
        iv. None of the above.

    b. Benadryl is a common anti-histamine drug that is used to treat allergies. Briefly explain why prolonged used of Benadryl could potentially inhibit immunity against bacterial infection.

    c. Briefly explain why prolonged used of Benadryl doesn't usually inhibit clearance of a bacterial infection.

    d. Recent studies have shown that polymorphisms of the histamine receptor have been linked to higher susceptibility to asthma and atopic dermatitis.
        i. Briefly explain how asthma/atopic dermatitis could be caused by a pathway linked to the immune system.

        ii. Given the role of histamine in the immune system, state whether each of the following statements is likely or unlikely regarding asthma/atopic dermatitis. Briefly explain your choice.
            - The polymorphic histamine receptor associated with asthma is sensitive to lower doses of histamine.

            - The polymorphic histamine receptor associated with asthma requires higher doses of histamine for activation.

- The polymorphic histamine receptor associated with asthma can be activated in the absence of histamine.

7. As stated in the video, “When lymphocytes detect cytokines, they respond by producing cytokines.” Explain why the immune system did not evolve to “cut out the middleman” (i.e., get rid of the lymphocyte step).

8. Imagine that you need to design a fail-safe mechanism to prevent chronic inflammation from causing massive tissue destruction in an organism.
    a. When would sensor cells “know” to initiate an anti-inflammatory response?

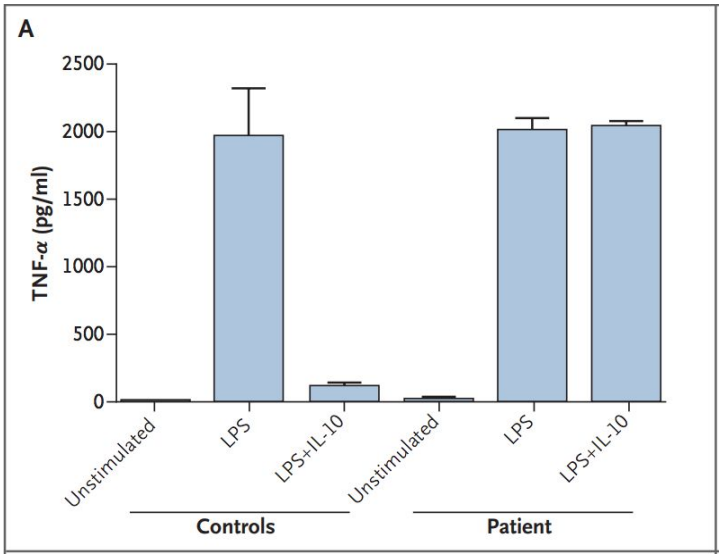
    b. Briefly describe how each of the following mechanisms could help sensors respond to chronic inflammation.
        i. Epigenetic changes (ie DNA methylation, histone modifications)

        ii. Transcriptional regulation

        iii. Post-translational modifications (ie phosphorylation)

    c. How would you expect sensor cells to respond after detecting chronic inflammation?

9. In the following graph, the inflammatory response of macrophages from a patient with inflammatory bowel disease (IBD) is shown next to the inflammatory response of macrophages from wild-type individuals. TNF-α is a common inflammatory cytokine.



   a. Compare and contrast the response to LPS and LPS+IL-10 between macrophages from controls and from the patient.

   b. What do you predict is the function of IL-10, generally?

   c. Given the data provided, predict what type of protein is likely defective in this patient.

10. From membrane fluidity to signaling molecules, steroids are active organic compounds that have many different biological functions. Certain steroids are used to treat patients with an overactive immune system, like patients with rash or asthma.
    a. Which phase would you expect steroids to induce - homeostasis, inflammation, or resolution?

    b. What could be the effect of using steroids in a normal individual?

c. Cortisol is a steroid hormone in humans that, among other functions, is released in response to stress. What would you expect to be the effect of high levels of stress in humans? Briefly explain.

Discussion Paper:
Zheng H., et al. (1995) Resistance to fever induction and impaired acute-phase response in interleukin-1 beta-deficient mice. Immunity. 3(1):9-19

Questions for Discussion Paper:

1. The authors of this paper use five different immune perturbations to assess the IL-1β KO mouse immune phenotype.
    a. Describe each of the methods they use. Include compound used, location of insult, length of trial, and experimental readout(s).

b. In your own words, what is each method attempting to evaluate? Try to answer this question in one sentence or less for each method.

c. The researchers observed a difference in response in one of the immune perturbations between KO and control mice, but no difference in response in the other manipulations.
    i. Which method provided a different immune response between the KO and WT mice?

    ii. How do the authors interpret this difference (i.e., what do they claim this tells you about the IL-1β KO mouse's immune system?)

2. Interleukin-1β (IL-1β) has been implicated in a broad spectrum of inflammatory responses and is regarded as a principal regulator of inflammation. It has been shown that treatment with neutralizing antibodies against IL-1β partially diminishes LPS-induced fever in adult mice. However, this paper shows that the IL-1β null mice are healthy and don't show any difference in response to LPS injection compared to WT mice (fever was statistically similar in both mice).
    a. Assuming that both studies used the same experimental approach with regards to the LPS injection, propose a hypothesis that explains the difference in the outcome. Briefly explain.

    b. How would you test your hypothesis? Briefly explain.

3. In Figure 4 of the paper, the authors show plasma IL-6 levels in KO and WT mice following turpentine treatment.
    a. What is the major finding from this figure?

b. What do you predict is the role of IL-6 in the acute-phase response?

c. Design an experiment to differentiate the roles of IL-1β and IL-6 in the acute-phase response in mice. Describe what conclusions can be made from the results.

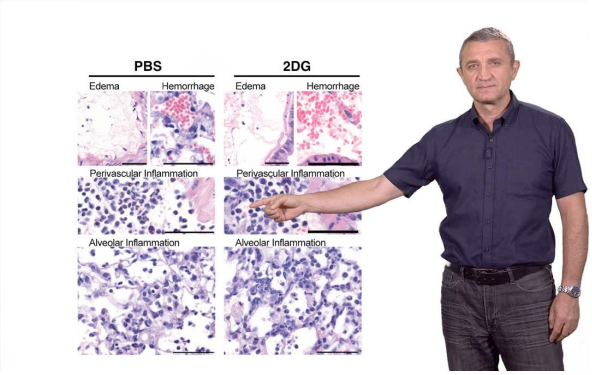
4. Taking into consideration what you learned from this paper, compare and contrast the role of the following cytokines upon LPS or Turpentine treatment in WT mice.
   a. TNF-α

   b. IL-6

   c. IL-1β

Second Video:
Title: Inflammation and Disease Tolerance: Surviving Acute Illness
Speaker: Ruslan Medzhitov
Time: 30:35

Concepts: Pathology of inflammation. Explains the connection between insulin/glucose homeostasis and the organismal response to viral and bacterial infections.



Questions for Second Video:

1. Compare and contrast the role of glucose in bacterial and viral infections. Include:
    a. How the consumption of glucose affects the outcome of an infection.

    b. The physiological pathways involved in each response.

2. You administer insulin to a mouse with sepsis. What effect would the insulin have in the mouse?
    a. The mouse will have higher chances of dying as insulin inhibits ketogenesis.
    b. The mouse will recover faster as insulin induces ketogenesis.
    c. You would not expect insulin to have any effect in the mouse recovery.
    d. The mouse will recover faster if given with a high glucose diet.
    e. None of the above.

3. State whether the following statements are true or false. If false, explain why.
    a. Anorexia is beneficial in viral infections.
    b. Evolutionarily it is not advantageous to fast upon viral infections.
    c. Decreased consumption of glucose helps the host fight a bacterial infection because it reduces food resources for the bacteria and inhibits bacterial growth.

4. A group in Africa drinks a special herbal tea that is supposed to help recovery from infection. You perform an experiment in mice where you compare the effect of drinking this tea to a control during *Listeria* infection and you obtain the results shown below.

   a. What effect does this tea have in mice? Briefly explain.

   b. Based on the findings from the Wang et al. study, in what molecular pathway would you expect compounds from this tea to be involved? Briefly explain.

   c. Propose an experiment to validate your hypothesis.

5. Prior to the study described in the video, the Medzhitov group identified synergistic lethality from co-infection with influenza and *Legionella* (bacterial infection) in mice (Reference: Jamieson et al., 2013). Based on the findings from the study described in the video, predict why this is the case.

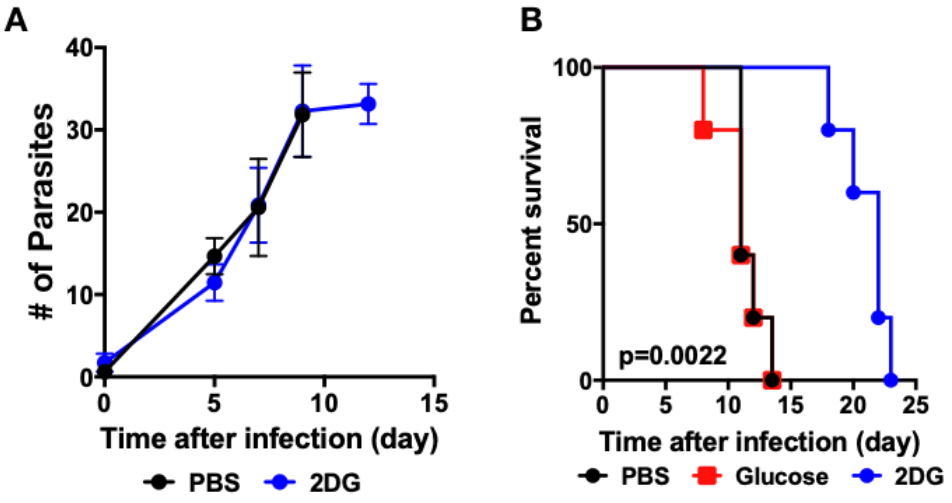
6. Components of the innate immune system are necessary for orchestrating the behavioral response to inflammation that Dr. Medzhitov describes. Imagine that you identify a new receptor, X, on peripheral nerve cells. When the gene for X is knocked out in mice, they no longer display common sickness behaviors such as fatigue and anorexia after infection with *Listeria* (bacterial infection).
    a. Based on what you know about innate immunity, what do you think this receptor binds to?

    b. How do you think binding of this ligand leads to anorexia and fatigue?

    c. How would you test your hypothesis *in vivo*?

7. Fasting metabolism is *not* protective in viral inflammation, yet mice infected with influenza reduce their chow consumption in the days immediately after infection. Predict why this might be so.

8. Sarah performed experiments in order to test the importance of host metabolism in malaria infections. She evaluated how PBS, 2DG and the Ketogenic diet affect mouse survival upon malarial infection. She quantified parasite burden in mouse blood and followed mouse survival, and obtained the results shown below.

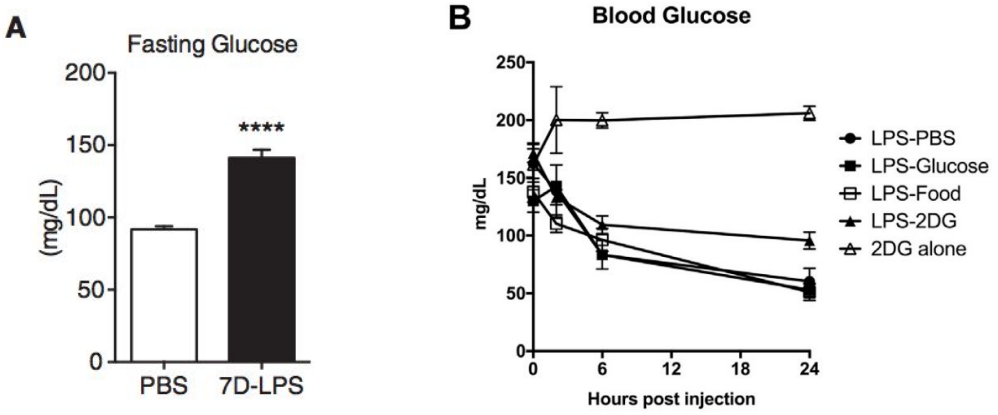


a. Do malarial infections resemble the results of bacterial or viral infections upon changes in the organismal metabolism? Briefly explain.

b. Sarah observed that the parasite count didn't change upon fasting. How can fasting help recovery if the count of parasites remains the same? Briefly explain.

c. What stereotypic behavior would you expect to observe in patients suffering malaria infections? Briefly explain.

9. Figure B below (right) is from the supplemental section of the Wang et. al 2016 paper described in the video. Figure A (left) is from a separate study (Okin & Medzhitov 2016). Figure B represents blood glucose levels over a 24-hour period post-injection, while Figure A represents blood glucose levels seven days after LPS injection.



(A) Blood glucose levels in mice given LPS for 7 days (7D-LPS) or mice receiving PBS as control (n = 10/condition). **(B)** Blood glucose at baseline, 2, 6 and 24 hr after 15 mg/kg IP LPS with PO gavage of PBS vehicle (LPS-PBS), glucose (LPS-Glucose), or Abbott Promote (LPS-Food) BID; or with IP 2DG (LPS-2DG). Blood glucose of mice treated with only 2DG IP are also shown.

a. Assuming that the conditions for LPS injection were identical, what does this new information tell you about the response to bacterial-induced inflammation?

b. Predict why blood glucose levels would respond in this way.

Answers for Session 2:

Questions for First Video:

1. Nonspecific (innate) immune responses include
    a. inflammation.
    b. antigen-antibody complexes.
    c. immunoglobulin action.
    d. complement and memory T cells.
    e. memory B cells.

2. Cytokines
    a. are regulatory Toll-like receptors.
    b. prevent the inflammatory response.
    c. include interferons and interleukins.
    d. are immunoglobulins.
    e. include interleukins and complement proteins.

3. State whether the following statements are true or false. If false, explain why.
    a. Cytokines and Chemokines are effectors. **False; they are mediators.**
    b. A Neutrophil is a Sensor cell. **False; neutrophils are effector cells.**
    c. Pathogens are required for activation of an inflammatory response. **False; the inflammatory response can be activated erroneously (i.e., allergies, autoimmunity).**

4. Compare and contrast the following sets of terms. Provide examples.
    a. Homeostatic state, stress response (physiological inflammation), and inflammation.
    All exist on a continuum of extremity, from homeostatic state <> stress response <> inflammation, and are related to the overall health of the host. The homeostatic state is the optimal physiological state of the host. The stress response is outside the bounds of the optimal (homeostatic) state, but within bounds that allow for normal function. Inflammation is further beyond the stress response, and can ultimately damage host tissues and impair function.

    b. Structural feature recognition and functional feature recognition.
    Structural feature recognition (i.e., pattern recognition receptors) recognizes specific parts of a pathogen while functional feature recognition (i.e., ER stress response) recognizes hallmarks of what the pathogen is actually doing inside the body. Both help the immune response "home in" on the type of pathogen so that it can mount an appropriate response.

5. The inflammatory pathway includes inducers, sensors, mediators, and effectors.
    a. Defend why sensors and effectors have evolved to be cell-based entities, while mediators are cell-free entities?
    This system has evolved because it is the most energetically favorable. The host must mount an immune response only when there is a real threat of infection. So, sensors and effectors must be able to integrate and interpret a range of inputs in order to launch an appropriate response (or not). This favors cells, which are sufficiently complex to take in many signals and determine whether they indicate a real threat or not. Because of this needed level of complexity, mediators have

evolved to be signaling molecules, which relay the decisions between sensors and effectors.

b. Imagine the opposite scenario and explain why it is less ideal.
If the opposite scenario were true, then the sensors and effectors wouldn't be complex enough to intake and properly interpret the range of signals needed to mount an appropriate response.

6. Histamine is produced by basophils and mast cells found in nearby connective tissues as a response to foreign pathogens. Histamine helps to fight infection by increasing the permeability of the capillaries to white blood cells and other proteins, which facilitates their entry to the infected tissue.
    a. The following characteristic defines the role of the basophil, histamine, and white blood cell, respectively:
        i. Effector, Mediator, and Sensor.
        ii. Mediator, Mediator, and Sensor.
        iii. Sensor, Mediator, and Effector.
        iv. None of the above.

    b. Benadryl is a common anti-histamine drug that is used to treat allergies. Briefly explain why prolonged used of Benadryl could potentially inhibit immunity against bacterial infection.
    By reducing the effect of the mediator (histamine), the immune system could stay in homeostasis even though there is a bacterial infection.

    c. Briefly explain why prolonged used of Benadryl doesn't usually inhibit clearance of a bacterial infection.
    This pathway is not the only immune response upon bacterial infections, i.e., histamine isn't essential for all anti-microbial responses in the body.

    d. Recent studies have shown that polymorphisms of the histamine receptor have been linked to higher susceptibility to asthma and atopic dermatitis.
        i. Briefly explain how asthma/atopic dermatitis could be caused by a pathway linked to the immune system.
        Asthma and atopic dermatitis could occur upon prolonged or aberrant activation of the immune system where the resolution part is delayed or doesn't occur (the system doesn't go back to homeostasis).

        ii. Given the role of histamine in the immune system, state whether each of the following statements is likely or unlikely regarding asthma/atopic dermatitis. Briefly explain your choice.
            - The polymorphic histamine receptor associated with asthma is sensitive to lower doses of histamine.
            Possible. The receptor would be hyper-sensitive and could be activated under not normal conditions.

- The polymorphic histamine receptor associated with asthma requires higher doses of histamine for activation.
  Unlikely. This system would likely return to homeostasis quicker.

- The polymorphic histamine receptor associated with asthma can be activated in the absence of histamine.
  Possible. Low levels of receptor activity could be activating an immunological response without the presence of pathogens, which would increase the chances of chronic inflammation.

7. As stated in the video, "When lymphocytes detect cytokines, they respond by producing cytokines." Explain why the immune system did not evolve to "cut out the middleman" (i.e., get rid of the lymphocyte step).
   Lymphocytes are the cells of the adaptive immune response. The adaptive response is an energetically demanding response to mount, so in the role Dr. Medzhitov describes, they function to integrate and interpret a host of signals before launching an adaptive response. This helps the host conserve energy by preventing unnecessary adaptive immune activation.

8. Imagine that you need to design a fail-safe mechanism to prevent chronic inflammation from causing massive tissue destruction in an organism.
   a. When would sensor cells "know" to initiate an anti-inflammatory response?
      Sensor cells would initiate an anti-inflammatory response once a certain threshold of pro-inflammatory signaling was reached.

   b. Briefly describe how each of the following mechanisms could help sensors respond to chronic inflammation.
      i. Epigenetic changes (ie DNA methylation, histone modifications)
         Epigenetic changes could turn on mediators (cytokines) to be released by the sensor cell, which would have an anti-inflammatory function.

      ii. Transcriptional regulation
         Like epigenetic changes, transcriptional regulation could turn on mediators (cytokines) to be released by the sensor cell, which would have an anti-inflammatory function.

      iii. Post-translational modifications (ie phosphorylation)
         Post-translational modifications (ie, phosphorylation of signaling molecules) could activate chronic stress signaling in sensor cells, which could lead to an anti-inflammatory response.

   c. How would you expect sensor cells to respond after detecting chronic inflammation?
      Upon detection of chronic inflammation, the sensor cell would turn off expression of inflammatory cytokines. (Or, express and secrete inhibitory cytokines that stop the inflammatory response).

9. In the following graph, the inflammatory response of macrophages from a patient with inflammatory bowel disease (IBD) is shown next to the inflammatory response of macrophages from wild-type individuals. TNF-α is a common inflammatory cytokine.

    a. Compare and contrast the response to LPS and LPS+IL-10 between macrophages from controls and from the patient.
    Response to LPS (initiation of inflammation) is the same, while response to LPS + IL-10 is impaired in the patient.

    b. What do you predict is the function of IL-10, generally?
    IL-10 is an anti-inflammatory cytokine (turns off TNF-α secretion).

    c. Given the data provided, predict what type of protein is likely defective in this patient.
    The most likely defect is in the IL-10 receptor. Alternatively, a downstream signaling protein of IL-10 receptor could be defective. (Reference: Inflammatory Bowel Disease and Mutations Affecting the Interleukin-10 Receptor (NEJM 2011) & Maloy & Powrie (Nature 2011): Intestinal homeostasis and its breakdown in inflammatory bowel disease)

10. From membrane fluidity to signaling molecules, steroids are active organic compounds that have many different biological functions. Certain steroids are used to treat patients with an overactive immune system, like patients with rash or asthma.
    a. Which phase would you expect steroids to induce - homeostasis, inflammation, or resolution?
    Resolution.

    b. What could be the effect of using steroids in a normal individual?
    Immunosuppressant. Person more likely to get sick.

    c. Cortisol is a steroid hormone in humans that, among other functions, is released in response to stress. What would you expect to be the effect of high levels of stress in humans? Briefly explain.
    High levels of stress would likely suppress the immune system and make people more susceptible to infections.

Discussion Paper:
Zheng H., et al. (1995) Resistance to fever induction and impaired acute-phase response in interleukin-1 beta-deficient mice. Immunity. 3(1):9-19

Questions for Discussion Paper:
1. The authors of this paper use five different immune perturbations to assess the IL-1β KO mouse immune phenotype.
    a. Describe each of the methods they use. Include compound used, location of insult, length of trial, and experimental readout(s).
    LPS - I.P. injection, 6 days after sensitization. Samples collected 3h after injection. Cytokine levels evaluated.

***Listeria* - Mice received I.V. injection of live bacteria. Mice were euthanized 4 days later and pathogen burden in spleen and liver was measured.**

Turpentine - Subcutaneous injection into left hindlimb. Body weight measured and mice euthanized at 0, 8, 24, 48, 72, and 96 hr after treatment. Blood collected from euthanized mice at each timepoint for IL-6 analysis. Liver tissue was collected from euthanized mice at each timepoint for acute-phase protein RNA expression. A separate group of mice were used to measure body temperature and activity.

Oxazalone - Application to stomach of mice followed by application to ear 5 days later. One day after the second exposure, mice were euthanized and swelling of ear was measured by weight.

mBSA - Three subcutaneous injections in the back followed by a subcutaneous injection in the left hind footpad 7 days later. One day after the second exposure, paw swelling was measured.

b. In your own words, what is each method attempting to evaluate? Try to answer this question in one sentence or less for each method.
LPS - systemic inflammatory response
***Listeria* - ability to clear infection**
Turpentine - localized acute inflammatory response
Oxazalone - delayed hypersensitivity
mBSA - delayed hypersensitivity

c. The researchers observed a difference in response in one of the immune perturbations between KO and control mice, but no difference in response in the other manipulations.
    i. Which method provided a different immune response between the KO and WT mice?
    Turpentine

    ii. How do the authors interpret this difference (i.e., what do they claim this tells you about the IL-1β KO mouse's immune system?)
    This tells them that the IL-1β KO mice have an impaired inflammatory response to acute, localized tissue injury. They also determined that these mice are resistant to fever and anorexia in response to the acute tissue injury. The KO mice appear to be normal in their ability to clear infection, mount a systemic inflammatory response, and mount an inflammatory response to a secondary exposure.

2. Interleukin-1β (IL-1β) has been implicated in a broad spectrum of inflammatory responses and is regarded as a principal regulator of inflammation. It has been shown that treatment with neutralizing antibodies against IL-1β partially diminishes LPS-induced fever in adult mice. However, this paper shows that the IL-1β null mice are healthy and don't show any difference in response to LPS injection compared to WT mice (fever was statistically similar in both mice).
    a. Assuming that both studies used the same experimental approach with regards to the LPS injection, propose a hypothesis that explains the difference in the outcome. Briefly explain.
    The IL-1β null mice could have other pathways that, during the course of development, compensate for the absence of IL-1β. In WT mice, however, these pathways are never activated, and therefore using neutralizing antibodies against IL-1β can have a noticeable effect.

    b. How would you test your hypothesis? Briefly explain.
    We know that other cytokines already have overlapping activities with IL-1β (e.g. TNF-α and IL-6), but these cytokines are induced to a similar level upon LPS treatment in WT and KO mice (basal levels are not shown). To evaluate other compensatory pathways, you could perform a microarray analysis to compare the differential expression of IL-1β KO mice versus WT. This could lead to the identification of additional pathway(s) that compensate in the developmental absence of IL-1β.

    Alternatively, you can use tamoxifen- inducible KO of IL-1β and perform the same experiment with an adult mouse.

3. In Figure 4 of the paper, the authors show plasma IL-6 levels in KO and WT mice following turpentine treatment.
    a. What is the major finding from this figure?
    The induction of IL-6 in the blood following localized, acute injury is impaired in IL-1β deficient mice.

    b. What do you predict is the role of IL-6 in the acute-phase response?
    IL-6 signaling in the blood likely helps activate and recruit immune cells to the site of injury. It may also help activate fever and anorexia.

    c. Design an experiment to differentiate the roles of IL-1β and IL-6 in the acute-phase response in mice. Describe what conclusions can be made from the results.
    Response to turpentine treatment (using readouts similar to those used in this paper) can be compared and contrasted in IL-1β KO, IL-6 KO, and IL-1β/IL-6 dKO mice. This experiment can tell you about the relative contributions of each cytokine in the acute phase response, and whether the cytokines are synergistic. To more closely evaluate the relative roles of each cytokine, researchers could use conditional genetic systems to "add back" each cytokine in turn following turpentine treatment.

    OR Could add back IL-6 in the same experimental setup and see if it is sufficient to rescue the acute phase response.

4. Taking into consideration what you learned from this paper, compare and contrast the role of the following cytokines upon LPS or Turpentine treatment in WT mice.
    a. TNF-α
    Is not induced by Turpentine, but is important for the LPS response.

    b. IL-6
    Induced by both Turpentine and LPS. In IL-1β null mice IL-6 is not activated. There might be a mechanism by which IL-1β is required for proper activation of IL-6 upon turpentine treatment.

    c. IL-1β
    Seems to have redundant activity with the LPS response pathway, but is required for the Turpentine-induced inflammatory response.

Questions for Second Video:

1. Compare and contrast the role of glucose in bacterial and viral infections. Include:
    a. How the consumption of glucose affects the outcome of an infection.
    Glucose consumption improves host survival upon viral infection and decreases survival upon bacterial (or sepsis) infection.

    b. The physiological pathways involved in each response.
    Activation of the fasting response (i.e., ketogenesis) is necessary for LPS (bacterial inflammation) survival. Glucose supplementation suppresses the fasting response. In viral infections, glucose is necessary for protein glycosylation in the ER to relieve ER stress. Both responses appear to be ways to help the organism tolerate, rather than control, the level of inflammation that occurs.

2. You administer insulin to a mouse with sepsis. What effect would the insulin have in the mouse?
    a. The mouse will have higher chances of dying as insulin inhibits ketogenesis.
    b. The mouse will recover faster as insulin induces ketogenesis.
    c. You would not expect insulin to have any effect in the mouse recovery.
    d. The mouse will recover faster if given with a high glucose diet.
    e. None of the above.

3. State whether the following statements are true or false. If false, explain why.
    a. Anorexia is beneficial in viral infections. **FALSE - Anorexia is detrimental.**
    b. Evolutionarily it is not advantageous to fast upon viral infections. **TRUE**
    c. Decreased consumption of glucose helps the host fight a bacterial infection because it reduces food resources for the bacteria and inhibits bacterial growth. **FALSE - It aids fight a bacterial infection because it activates ketogenesis, which aids the bodily response to infection.**

4. A group in Africa drinks a special herbal tea that is supposed to help recovery from infection. You perform an experiment in mice where you compare the effect of drinking this tea to a control during *Listeria* infection and you obtain the results shown below.
    a. What effect does this tea have in mice? Briefly explain.
    This tea is detrimental for the recovery of mice that experience a bacterial infection.

    b. Based on the findings from the Wang et al. study, in what molecular pathway would you expect compounds from this tea to be involved? Briefly explain.
    A component of this tea is likely to be involved in the fasting metabolic pathway. This tea could induce insulin (simulates high insulin/ high glucose conditions). Alternatively, the tea could inhibit ketogenesis in an insulin-independent manner.

    c. Propose an experiment to validate your hypothesis.
    Measure levels of PPARα and FGF21 after tea consumption. Plasma levels of PPARα and FGF21 should decrease in mice treated with tea compared with control (PBS) mice. Could also measure blood insulin/glucose levels after tea administration.

5. Prior to the study described in the video, the Medzhitov group identified synergistic lethality from co-infection with influenza and *Legionella* (bacterial infection) in mice (Reference: Jamieson et al., 2013). Based on the findings from the study described in the video, predict why this is the case.
The metabolic responses to bacterial and viral infection (at least those uncovered in the Wang et al. 2016 paper) appear to be fundamentally at odds with one another. That is, during infection with *Legionella,* it is advantageous for mice to induce fasting metabolism and reduce the amount of blood glucose, while during infection with influenza, it is advantageous for sufficient blood glucose levels to relieve UPR stress in the brain.

6. Components of the innate immune system are necessary for orchestrating the behavioral response to inflammation that Dr. Medzhitov describes. Imagine that you identify a new receptor, X, on peripheral nerve cells. When the gene for X is knocked out in mice, they no longer display common sickness behaviors such as fatigue and anorexia after infection with *Listeria* (bacterial infection).
    a. Based on what you know about innate immunity, what do you think this receptor binds to?
    Receptor X likely binds to an inflammatory cytokine such as IL-1, IL-6, or TNF that is secreted upon bacterial infection.

    b. How do you think binding of this ligand leads to anorexia and fatigue?
    This activates the peripheral neuron to send signals to the brain, which leads to the change in behavior (the nerve cells in question may in fact be the vagus nerve).

    c. How would you test your hypothesis *in vivo*?
    Multiple answers may apply: express mutant receptors with inhibited binding for specific cytokines; use cytokine KO or conditional expression mice for the study. Could also inhibit the ability of the peripheral nerve to communicate with the brain.

7. Fasting metabolism is *not* protective in viral inflammation, yet mice infected with influenza reduce their chow consumption in the days immediately after infection. Predict why this might be so.
   Although there is no clear answer for this, students might predict that reduction of food consumption could be an evolutionary artifact (i.e., that anorexia is more often than not helpful rather than harmful during infection). It could also be viral-titer dependent: In the experiment for Figure 3A (from Wang et al, Cell, 2016), mice were given a sub-lethal dose, while in the experiment for Figure 3B, they were given a lethal dose of influenza.

8. Sarah performed experiments in order to test the importance of host metabolism in malaria infections. She evaluated how PBS, 2DG and the Ketogenic diet affect mouse survival upon malarial infection. She quantified parasite burden in mouse blood and followed mouse survival, and obtained the results shown below.
   a. Do malarial infections resemble the results of bacterial or viral infections upon changes in the organismal metabolism? Briefly explain.
      Malaria infection behaves similar to bacterial infections where fasting seems to be beneficial.

   b. Sarah observed that the parasite count didn't change upon fasting. How can fasting help recovery if the count of parasites remains the same? Briefly explain.
      Inflammation, along with other pathways, is activated upon infection which results in a loss of homeostasis. It is possible that fasting helps the system recover homeostasis faster. This process can be pathogen-burden-independent.

   c. What stereotypic behavior would you expect to observe in patients suffering malaria infections? Briefly explain.
      Anorexia. It would be evolutionarily advantageous.

9. Figure B below (left) is from the supplemental section of the Wang et. al 2016 paper described in the video. Figure A (right) is from a separate study (Okin & Medzhitov 2016). Figure B represents blood glucose levels over a 24-hour period post-injection, while Figure A represents blood glucose levels seven days after LPS injection.
   a. Assuming that the conditions for LPS injection were identical, what does this new information tell you about the response to bacterial-induced inflammation?
      This new information suggests that ketogenesis is not required during the entirety of bacterial inflammation (at least, if inflammation is prolonged). In fact, the figure on the right indicates that elevated blood glucose may be advantageous after prolonged inflammation.

   b. Predict why blood glucose levels would respond in this way.
      This may be because immune cells favor glycolysis - as the adaptive response is ramping up, the body needs to provide more glucose. One major caveat: there is a 5-day gap (days 2-6) that are unaccounted for by this data. It could help to give a more granular view of what is going on. References: Wang et al 2016 paper; Okin & Medzhitov 2016 paper