

Session 4: Molecular View of Adaptive Immunity

Overview:

This session provides a molecular overview of T cell receptor (TCR) activation and signaling. It shows how TCRs bind to their corresponding peptide:MHC complexes to form the immunological synapse. As shown in this session, the stability and strength of the immunological synapse correlates with the activation of the TCR signaling cascade. TCR activation is mediated by the integration of convergent signals from antigen presenting cells, including the activation of Pattern-Recognition Receptors and the presentation of antigen via MHC molecules.

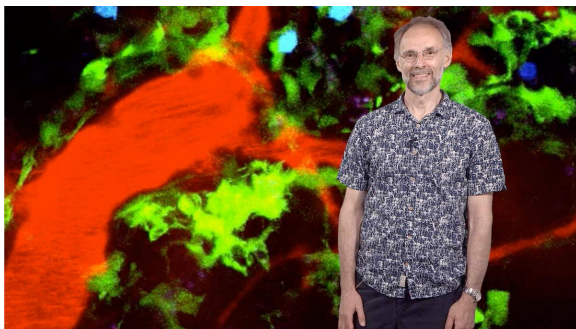
First video:

Title: The Immunological Synapse: Antigen Recognition

Speaker: Michael Dustin

Time: 20:07

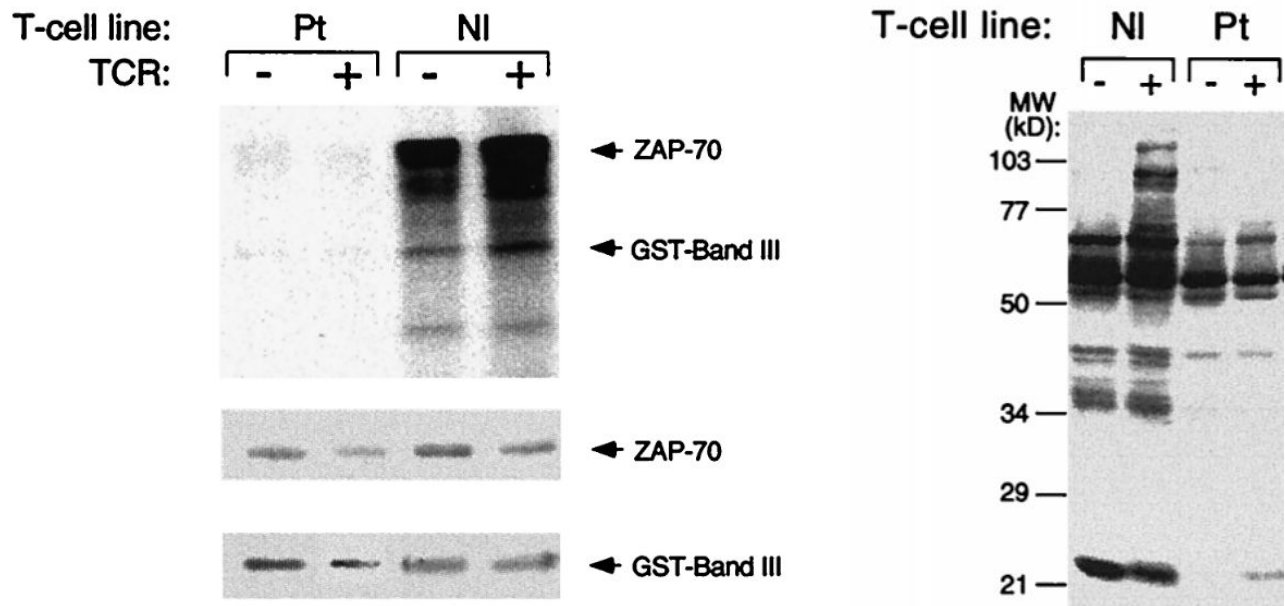
Concepts: T cell receptor (TCR) activation and signaling cascade, components of the immunological synapse



Questions for First Video:

1. State whether the following statements are true or false. If false, explain why.
 - a. Binding of MHC-complex and TCR activates T cells.
 - b. Stable immunological synapses occur during T cell activation.
 - c. If the phosphorylation of the TCR by Lck is inhibited, T cells cannot be activated.
 - d. Specificity in the immunological synapse is conferred by the TCR.
2. Following interaction with a mature dendritic cell, an activated T cell typically follows one of two fates.
 - a. Name the two locations an activated T cell can migrate to.
 - b. What is the role of the T cell in each location?

5. Scientists studied a patient that had a unique mutation in the protein tyrosine kinase, ZAP-70 (Elder M., et al. (2001) J Immunol).



RIGHT: Tyrosine phosphoprotein cascade: Patient (Pt) and normal (NI) T cell lines were studied. Unstimulated (-) and stimulated (+) lysates were analyzed by 4G10 (anti-phosphotyrosine mAb) immuno-blot.

LEFT: ZAP-70 catalytic activity. Whole cell lysates of patient (Pt) and normal (NI) T cell lines were subjected to an in vitro kinase assay. Unstimulated (-) and stimulated (+) lysates.

- Given the data shown above, what is the effect of this mutation on the tyrosine phosphoprotein cascade. Briefly explain.
- Compare and contrast the expression and catalytic activity of ZAP-70 in the patient and in WT individuals. What conclusion can be made from these data?
- Given the effect of this mutation, what type of symptoms should this patient have? Briefly explain.

c. CD4:

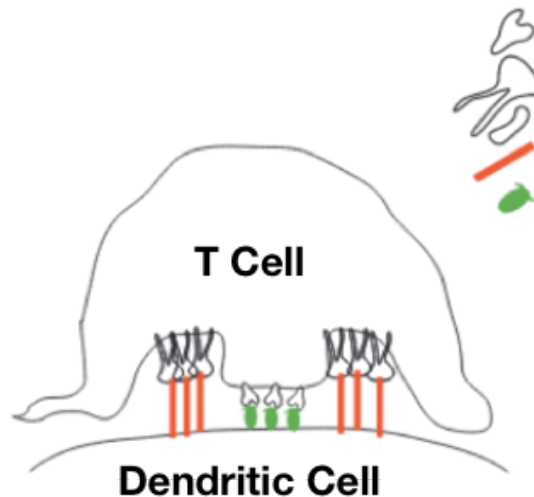
10. For proper TCR activation, the immunological synapse formation requires a dynamic movement of proteins that differ in localization, timing, and function.

a. Fill out the blanks on the table:

Protein/Protein Complex	Cellular localization	Function
MHC		
LAF-1		
ZAP70		
Lck		
CD45		
CD28		
CD80/86 (B7-1/B7-2)		
CTLA-4		

b. Shown below is a diagram showing the immunological synapse between a dendritic cell and a helper-T cell.

i. Identify the molecules shown in the diagram below. Consider the following proteins: MHC, TCR, Lsk, CD4, CD8, ICAM-1, LFA-1, ZAP70, and CD45.



- ii. Where would you expect to find the proteins considered in (i) that were not present in the diagram? What is the function of these proteins? Briefly explain.

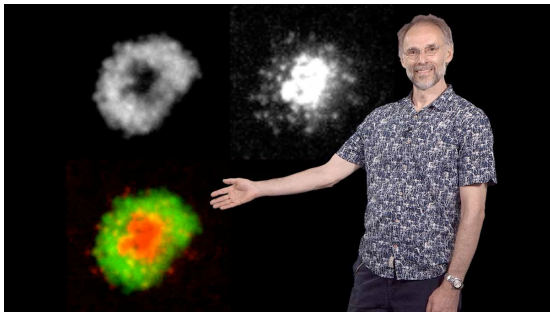
Second video:

Title: The Immunological Synapse: Signaling and Function

Speaker: Michael Dustin

Time: 30:18

Concepts: Immunological synapse, role of CD45 in the activation of the immunological synapse, cancer-immunotherapy, function of actin/WASp in the immunological synapse, and synapse vs kinapse

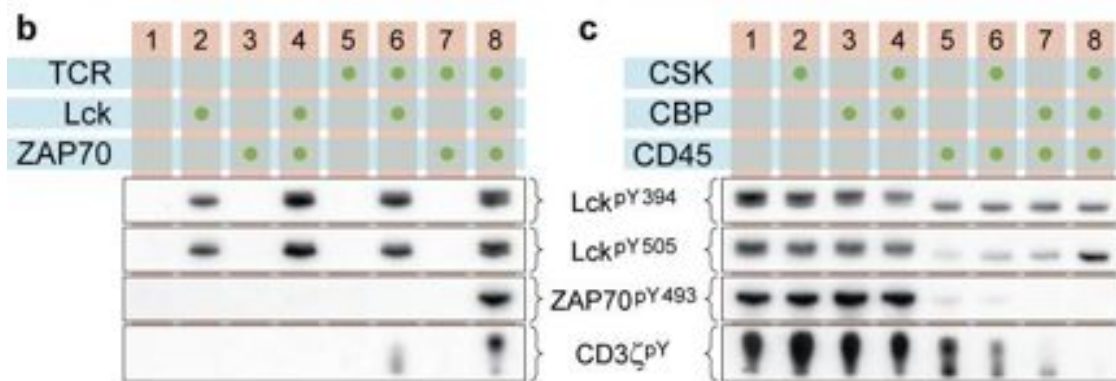


Questions for Second Video:

1. Indicate whether the following statements are true or false. If false, briefly explain your answer.
 - a. T cells involved in a kinapse can send more cytotoxic molecules to infected cells than T cells involved in a synapse.
 - b. Actin drives immunological synapse formation.
 - c. During T cell activation, CD45 is recruited to the immunological synapse.

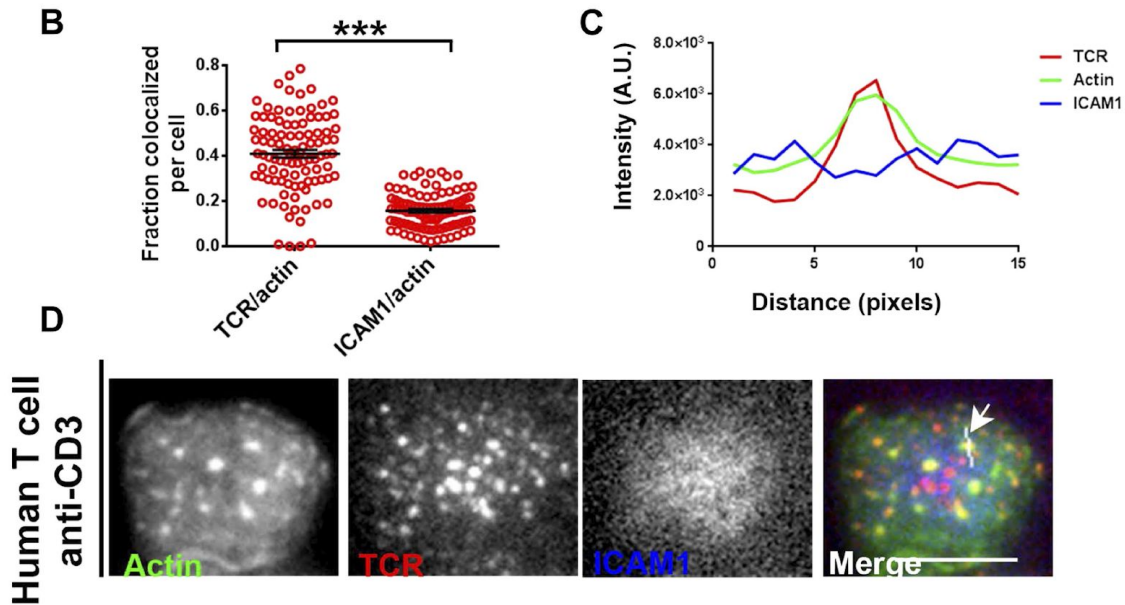
5. In order to study the minimal requirement for an immunological synapse, the Vale lab (James & Vale (2012) Nature) reconstituted in HEK (kidney) cells the proteins involved in the synapse.

Shown below on (b) is the western blot of phosphorylated proteins after transfection of HEK cells with selected molecules (green circles). On (c), cells transfected with the TCR, Lck and ZAP70, were transfected with additional molecules (green circles). CD3 ζ phosphorylation is used as a marker for TCR activation.



- a. What proteins are required for CD3 ζ phosphorylation?
- b. What is the potential role of CSK, CBP, and CD45 in the signaling pathway of TCR? Briefly explain.
- c. Given this result, how would you classify the phosphorylation of ZAP70? Activating or inhibiting?
6. Researchers found that inhibition of CTLA-4, a T cell surface protein involved in synapses, led to tumor shrinkage in mouse models of cancer.
- a. What is the function of CTLA-4?
- b. How could inhibition of CTLA-4 promote anti-tumor immunity?

- c. Anti-CTLA-4 therapy failed in some models of cancer. Briefly explain why.
- d. Researchers later determined that combining anti-CTLA-4 therapy with ionizing radiation could rescue the antitumor effects CTLA-4 inhibition. Based on your understanding of the immune system, predict why this is the case.
7. The structure of the immunological synapse plays an important role in its function.
- Predict why the large, central cluster of T-cell receptors (TCRs) in an immunological synapse is not active in signaling, while the peripheral TCRs are.
 - Predict why the phosphatase CD45 is excluded from TCR signaling clusters.
8. Researchers sought to understand how the actin cytoskeleton interacts with components of the immunological synapse. TCR = T cell receptor. ICAM1 = adhesion molecule expressed by T cells. (Reference: Elife. 2015 Mar 11;4. doi: 10.7554/eLife.04953)



Answers for Session 4:

Questions for First Video:

1. State whether the following statements are true or false. If false, explain why.
 - a. Binding of MHC-complex and TCR activates T cells. **(False; you also need binding of CD8/CD4, and binding of CD28 to B7)**
 - b. Stable immunological synapses occur during T cell activation. **(True)**
 - c. If the phosphorylation of the TCR by Lck is inhibited, T cells cannot be activated. **(True)**
 - d. Specificity in the immunological synapse is conferred by the TCR. **(True)**

2. Following interaction with a mature dendritic cell, an activated T cell typically follows one of two fates.
 - a. Name the two locations an activated T cell can migrate to.
B cell follicle in lymph node or sites of inflammation in periphery.

 - b. What is the role of the T cell in each location?
In the follicle, T helper cells help instruct naive B cells to mature and to increase the specificity of the BCR. In the periphery, they help to fight infection by serving cytotoxic or helper T effector functions.

 - c. Compare and contrast T cell:dendritic cell and T cell:B cell interactions.
Both are part of adaptive immunity and lead to a highly specific immune response. When naive T cells interact with dendritic cells, it can lead to T cell activation/maturation. When activated T cells interact with naive B cells in the follicle, it can lead to B cell activation and help increase the specificity of the BCR.

3. Activation of the T cell receptor (TCR) by the MHC complex can be classified as strongly activating (agonist), weakly activating (weak agonist), or inactive (null). The wide range of biological activities displayed by altered peptide ligands has been correlated with the half-life of the TCR–MHC-peptide interaction.
 - a. How would you classify a perfect match between the antigen in the MHC complex and the TCR?
This should be a strong activating interaction.

 - b. Antigen B is a perfect match to a TCR; what would happen to the TCR-MHC peptide interaction if you were to change one of the amino acids in antigen B? Briefly explain.
Unless the amino acid is replaced by another with similar properties, interaction should weaken and signaling should weaken. TCR-MHC-peptide interaction should have a shorter half-life.

 - c. Upon strong activation, clustering of TCR-MHC complexes is observed. How can this clustering secure activation of T cells? Briefly explain.
Clustering stabilizes an interaction. Similar to velcro, more consecutive interactions will decrease the chances of the two cells separating from each other and therefore will increase signaling through the TCR.

4. Four specific challenges to T cell activation are listed in the video.
- Name two of the challenges and briefly describe.
TCR and pMHC are small - relative to the size of the cells, the two surface molecules are small. This makes it hard for the molecules to “find” one another.

pMHC rare - Hundreds, if not thousands, of antigen are presented by a dendritic cell (DC) at any given time. This means that the odds of the TCR finding its specific match are low.

TCR affinity for pMHC is low - Even if the TCR finds its cognate pMHC, the binding between the two molecules is not strong enough to facilitate a long-term interaction.

T cell and DC are moving - This too can prevent the TCR and pMHC from developing a strong interaction.
 - How do T cells overcome these challenges?
The T cell overcomes these challenges by activating adhesion molecules, which bind to adhesion molecules on the surface of DCs to increase the strength and longevity of the TCR:pMHC interaction.
 - Why would TCR:pMHC binding be weaker than BCR:antigen binding?
Ultimately, the TCR:pMHC interaction will be released so that activated T cells can go on to complete their effector functions. BCR:antigen binding, on the other hand, is an effector function itself (binding of antibodies to pathogen aids pathogen recognition and clearing) and so the bond is much stronger.
5. Scientists studied a patient that had a unique mutation in the protein tyrosine kinase, ZAP-70 (Elder M., et al. (2001) J Immunol).
- Given the data shown above, what is the effect of this mutation on the tyrosine phosphoprotein cascade. Briefly explain.
This patient does not seem to have a functional tyrosine phosphoprotein cascade. In normal cells, you can observe upon activation several proteins being phosphorylated. This is absent in the patient cells.
 - Compare and contrast the expression and catalytic activity of ZAP-70 in the patient and in WT individuals. What conclusion can be made from these data?
Although ZAP-70 is still expressed in patients at a similar level to WT individuals, this mutation seems to inactivate the catalytic activity of ZAP-70.
 - Given the effect of this mutation, what type of symptoms should this patient have? Briefly explain.
This patient should have Severe Combined Immunodeficiency (SCID) (or severe compromised immune system) as T cells cannot be activated upon antigen recognition. The signaling cascade is blocked as ZAP-70 is not able to catalyze the phosphorylation reaction required for proper signaling.

6. Mature dendritic cells in the lymph node interact with thousands of T cells each hour.
 - a. Why is this necessary?
Numerous interactions must occur before each cell finds its antigen-specific “match”.
 - b. In the immunological synapse, which molecular interaction between T cells and dendritic cells (DCs) is the most important? Why?
The TCR:pMHC interaction is the most important because it ensures that a pathogen-specific immune response is mounted (i.e., that the right type of TCR is activated).
 - c. Briefly explain why a single dendritic cell is able to interact with T cells with different TCRs.
Each T cell is specific to only one antigen, while each DC presents hundreds to thousands of different antigen at its surface.

7. In secondary lymphoid tissues, B cells get information about infection from macrophages while T cells get information about the same infection from dendritic cells. How does the immune system ensure that lymphocytes synergize to attack the same pathogen?
After activation, some T cells (helper T cells) enter the B cell follicle of the lymph node to aid their activation and increase their specificity for the pathogen. This is one way that the two types of lymphocytes “cross-talk” with one another to lead to optimal adaptive immune activation.

8. T cell receptor activation is regulated both spatially and temporally.
 - a. Give specific examples of each type of regulation in T cell activation *at the molecular level*.
**Spatial = “bullseye” organization of immunological synapse
Temporal = TCR activation requires simultaneous activation of coreceptors and inhibition of negative regulation**
 - b. Why is each type of regulation necessary?
The spatial organization allows for activation and binding of adhesion molecules at the periphery of the TCR:pMHC interaction, which increases the longevity of the T cell:DC interaction. Temporal regulation is key to ensure that only pathogen-specific T cells are activated (i.e., to avoid autoimmunity or allergy).

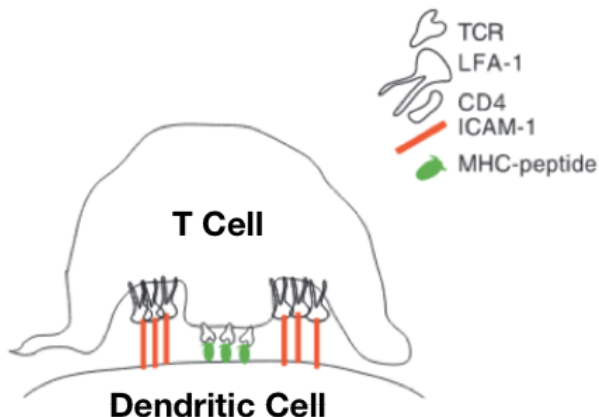
9. For the following proteins, briefly explain their role in the immunological synapse.
 - a. LFA-1:
Present in T cells. Binds to ICAM protein to stabilize the immunological synapse during T cell activation.
 - b. MHC complex:
Present in antigen presenting cells. Contains the antigen that will interact with the T cell receptor. Without this specific interaction, the immunological synapse is transient. If the TCR is able to bind to the antigen, synapse becomes stable.

- c. CD4:
After binding between MHC and TCR, CD4 will be close enough to interact in an antigen-independent manner with the MHC complex and activate a signaling cascade that will stabilize the immunological synapse and activate the T cell.

10. For proper TCR activation, the immunological synapse formation requires a dynamic movement of proteins that differ in localization, timing, and function.
 a. Fill out the blanks on the table:

Protein/Protein Complex	Cellular localization	Function
MHC	Membrane. Center of synapse	Present in APCs. Present antigen to T-helper cells (MHC-II) or cytotoxic-T cells (MHC-I)
LFA-1	Membrane of T cells. Surrounds the synapse	Help stabilization of synapse. Binds to adhesion protein in APCs
ZAP70	Kinase. Intracellular protein. Recruited to synapse upon activation of T cells	Activates the TCR signaling cascade
Lck	Kinase. Localized to synapse	Activates the TCR signaling cascade
CD45	Phosphatase	Inhibits TCR signaling
CD28	Membrane of T cells	Receives signal from CD80/86 and activates TCR
CD80/86 (B7-1/B7-2)	Membrane of dendritic cells (or APCs)	TMP that transduces signaling from Pattern-recognition receptor (PRR) (like Toll-Like Receptor) to activate TCR. It's the ligand of CD28
CTLA-4	Intracellular protein	Inhibits T cell proliferation

- b. Shown below is a diagram showing the immunological synapse between a dendritic cell and a helper-T cell.
 i. Identify the molecules shown in the diagram below. Consider the following proteins: MHC, TCR, Lsk, CD4, CD8, ICAM-1, LFA-1, ZAP70, and CD45.



- ii. Where would you expect to find the proteins considered in (i) that were not present in the diagram? What is the function of these proteins? Briefly explain.
Lsk: T cell kinase localized to the intracellular region of the immunological synapse upon activation of TCR. Lsk activates TCR signaling cascade.

CD4: T-helper cell protein localized next to the TCR and aids TCR activation.

CD8: Should not be present as this is a cytotoxic-T cell protein. Aids TCR activation in cytotoxic-T cells.

ZAP70: T cell kinase localized to the intracellular region of the immunological synapse upon activation of TCR.

CD45: Phosphatase present in T cells. Should be excluded from the immunological synapse upon TCR activation.

Questions for Second Video:

1. Indicate whether the following statements are true or false. If false, briefly explain your answer.
 - a. T cells involved in a kinapse can send more cytotoxic molecules to infected cells than T cells involved in a synapse. **(False – Synapse sends the most cytotoxic molecules)**
 - b. Actin drives immunological synapse formation. **(True)**
 - c. During T cell activation, CD45 is recruited to the immunological synapse. **(False – CD45 is excluded from the synapse)**

2. Five ways in which the TCR can be activated are described in the video.

- a. Name two and briefly describe how they lead (or *likely* lead) to TCR signaling.
CD45/phosphatase exclusion - prevents negative regulation of phosphorylation

Inhibition of Csk - Csk is a negative regulator, so inhibition of Csk means inhibition of negative regulation

Conformational change - likely leads to change in intracellular signaling strength and/or ability

Release of cytoplasmic domains from sequestration by lipids - likely enables cytoplasmic domains of the TCR to function and send signals

Force induced catch bonds - increases strength of TCR binding to pMHC and therefore increases TCR signaling

- b. Explain why it is necessary to have so many levels of regulation.
It is very important to ensure that only pathogen-specific T cells are activated, otherwise aberrant T cell activation could lead to allergy or autoimmunity. Therefore, the adaptive immune system has evolved several levels of regulation to make sure that only the necessary T cells are activated for a particular context.

3. The immunological synapse serves both activation and effector functions. Compare and contrast each of these roles.
Structurally, the synapse looks similar for both of these functions (has the typical “bullseye” shape). In synapses that serve an activation role, the interaction is between a naive T cell and a dendritic cell and the result is T cell activation. In synapses that serve an effector function, the interaction is between a mature T cell and a target cell (i.e., a virus-infected cell) and one result can be target cell killing, for example through granule secretion.

4. Wiscott Aldrich Syndrome protein (WASP) deficiency results in defects in overall T cell activation. The activation of WASP generates a dynamic F-actin architecture in the context of the immunological synapse.
 - a. Briefly explain the role of actin in the formation of the immunological synapse.
Actin helps in the formation of the immunological synapse. Also, F-actin foci are associated with TCR signal amplification.

 - b. For the following processes, briefly describe the effect of WASP deficiency.
 - i. Immunological Synapse: **No effect. T cells missing WASP can construct immunological synapses.**

 - ii. Actin Foci Formation: **Reduced number of actin-Foci, which recruits crucial proteins for the signal transduction upon TCR activation. As a result, these cells fail to respond to infected cells properly.**

5. In order to study the minimal requirement for an immunological synapse, the Vale lab (James & Vale (2012) Nature) reconstituted in HEK (kidney) cells the proteins involved in the synapse.

Shown below on (b) is the western blot of phosphorylated proteins after transfection of HEK cells with selected molecules (green circles). On (c), cells transfected with the TCR, Lck and ZAP70, were transfected with additional molecules (green circles). CD3 ζ phosphorylation is used as a marker for TCR activation.

- a. What proteins are required for CD3 ζ phosphorylation?
You need TCR, Lck, and ZAP70 in order to activate CD3 ζ via phosphorylation.

- b. What is the potential role of CSK, CBP, and CD45 in the signaling pathway of TCR? Briefly explain.
These proteins seem to have a synergistic action to restrain Lck and/or ZAP70. This inhibition also inhibits signaling of TCR as shown by the reduction of CD3 ζ phosphorylation.

- c. Given this result, how would you classify the phosphorylation of ZAP70? Activating or inhibiting?
Activating.

6. Researchers found that inhibition of CTLA-4, a T cell surface protein involved in synapses, led to tumor shrinkage in mouse models of cancer.
- What is the function of CTLA-4?
CTLA-4 is a negative regulator of TCR signaling. It acts as an "off" switch when bound to CD80 or CD86 on the surface of antigen-presenting cells. CD80/86 signaling is important for the Pattern Recognition Receptor (e.g. Toll-like Receptors) mediated activation of TCR.
 - How could inhibition of CTLA-4 promote anti-tumor immunity?
By blocking CTLA-4's inhibitory function, T cells with TCRs that recognize tumor antigen are more likely to be activated and target tumor cells.
 - Anti-CTLA-4 therapy failed in some models of cancer. Briefly explain why.
Although treatment with anti-CTLA-4 can increase TCR signaling, it decreases synapse stability (destabilizes the immunological synapse), leading to a reduction in overall activation in some cases.
 - Researchers later determined that combining anti-CTLA-4 therapy with ionizing radiation could rescue the antitumor effects CTLA-4 inhibition. Based on your understanding of the immune system, predict why this is the case.
Ionizing radiation could be activating the innate immunological responses, leading to a stronger priming of T cells and stronger immunological synapses.
7. The structure of the immunological synapse plays an important role in its function.
- Predict why the large, central cluster of T-cell receptors (TCRs) in an immunological synapse is not active in signaling, while the peripheral TCRs are.
The large central cluster likely aids in anchoring the T cell to the dendritic cell in an antigen-specific manner. These TCRs may be linked on their cytoplasmic side to the cytoskeleton, which may interfere with signal transduction.
 - Predict why the phosphatase CD45 is excluded from TCR signaling clusters.
The TCR activation signal is transduced intracellularly by kinases (via phosphorylation of downstream targets). CD45 removes phosphorylation, therefore acts as a negative regulator of TCR signaling. When the TCR is activated, CD45 is excluded from the clusters so that it cannot turn the signaling pathway "off".
8. Researchers sought to understand how the actin cytoskeleton interacts with components of the immunological synapse. TCR = T cell receptor. ICAM1 = adhesion molecule expressed by T cells. (Reference: Elife. 2015 Mar 11;4. doi: 10.7554/eLife.04953)
- In the figure above, Panel B represents quantitation of the fraction of TCR or ICAM1 molecules colocalized with actin clusters at immunological synapses. What is the take-home message of this figure?
The TCR more frequently colocalizes with actin at immunological synapses than ICAM1 does.

- b. In the figure above, Panels C and D show data from imaging actin, TCRs, and ICAM1 at the immunological synapse of a single T cell. The thin white line marked by the arrow in the 'merge' panel of D is the line plotted in C, where relative intensities of the signal for each molecule were quantified across a single TCR microcluster. What is the take-home message of these figures?
TCR/actin colocalization increases at a TCR microcluster, while ICAM1/actin colocalization does not.
- c. Compare and contrast the information provided by Panel B vs. Panels C/D.
The figure in Panel B generally tells us that the TCR more frequently associates with actin than ICAM1 does at immunological synapses. However, it doesn't provide spatial details. The figures in C and D tell us that the TCRs and actin more strongly colocalize at TCR microclusters whereas ICAM1/actin colocalization is more diffuse.
9. Immunological kinapses are related to, but distinct from, the immunological synapse.
- a. Compare and contrast an immunological synapse with an immunological kinapse.
Structurally, they are similar. The TCR is clustered in the middle, while adhesion molecules are dispersed around the TCR cluster. However, unlike a synapse which has a typical bullseye organization, kinapses exhibit a broken or incomplete bullseye. Synapse is a more stable interaction. Synapses result in stronger signaling cascade and proper activation of T cells. Kinapse is more transient and doesn't have strong activation of TCR. Kinapses are associated with autoimmunity and cancer.
- b. Self-reactive T cells favor kinapse formation over synapse formation. Predict how this contributes to autoimmunity.
The formation of a kinapse may result in incomplete or aberrant signaling to a naive T cell, which means that the T cell isn't activated or inactivated properly. In the case of autoimmunity, it is likely that kinapse formation makes self-reactive T cells harder to regulate.
- c. Based on your understanding of the role of kinapses in disease, how would you design a therapy to treat cancer and/or autoimmunity?
One approach could be a drug that stabilizes the cytoskeleton and limits lamellopodia turnover so that a proper synapse can form. Alternatively, a drug that increases WASp kinase signaling or inhibits PKC-theta kinase signaling could favor synapse formation.

Questions for Discussion Paper:

1. This paper investigates the expression and localization of two T cell surface proteins, CD28 and CTLA-4, following T cell stimulation by antigen-presenting cells.
- a. Briefly describe the function of each protein.
CD28 sends an activation signal to the T cell, resulting in clonal expansion. CTLA-4 sends an inhibition signal to the T cell, resulting in restriction of T cell expansion.

- b. What protein do they recognize on the surface of antigen-presenting cells?

B7

- c. Predict the consequence of TCR:pMHC binding in the absence of costimulation by either CD28 or CTLA-4.

In the absence of any costimulatory signal whatsoever, the T cell will become anergic (unresponsive).

2. Activation of naive T cells requires more than just TCR activation. Following pattern-recognition receptor binding to pathogen associated molecular patterns, dendritic cells increase their expression of CD80/86, the ligand of CD28 and CTLA-4 on T cells. Binding of CD28 to CD80/86 enhances T cell maturation, but CTLA-4 has inhibitory effects.

- a. Are the expression and localization of CD28 and CTLA-4 the same or different in naive T cells? Briefly explain.

CD28 is constitutively expressed in the membrane of naïve T cells. In contrast, CTLA-4 protein is not expressed in naïve cells, but expression increases upon T cell activation.

- b. Compare and contrast the expression and localization of CD28 and CTLA-4 following TCR:pMHC binding.

Following T cell stimulation by TCR:pMHC interaction, both CD28 and CTLA-4 rapidly translocate to the site of the immunological synapse. CD28 appears to be localized to the plasma membrane during this process, while CTLA-4 is either adjacent to the microtubule organizing complex (MTOC) on the intracellular side of the synapse or at the plasma membrane (PM), depending on the strength of the TCR signal. CTLA-4 total expression stays constant following TCR:pMHC binding. The authors do not show data for CD28 expression.

- c. Based on your understanding of the function of each of these proteins, explain why the researchers observed differences in their localization relative to the immunological synapse following TCR:pMHC binding.

It appears that CD28 translocalizes to the PM following TCR:pMHC binding by default, to provide activating signals. CTLA-4, on the other hand, appears to be at the ready to provide “stop” signals in case the T cell should not be activated (i.e., if it is self-reactive).

- d. This paper shows that CTLA-4-mediated inhibition correlates with the strength of TCR activation. Briefly hypothesize why.

Possible negative feedback loop to prevent aberrant TCR activation.

- e. Why would CTLA-4 but not CD28 respond differentially to the strength of the TCR:pMHC interaction?

TCR binding to the wrong antigen can be a bad thing, especially if the antigen is from the body. The immune system has evolved to dampen the adaptive response of lymphocytes that bind their antigen too strongly, almost as a fail-safe mechanism. It follows that CTLA-4 (which is inhibitory) would be active at the immunological synapse when the TCR binds its antigen too tightly. It also appears that the adaptive immune system has evolved to have CD28 provide a default, “go” signal that can be overridden by CTLA-4.

- f. Explain why antibodies against CTLA-4 are used as a cancer therapy.

Inhibiting the negative feedback loop will keep synapses and TCR signaling activated longer, which could enhance the recognition of cancer cells by the immune system.

3. (Optional Discussion) Compare and contrast the authors' use of the term “activation” with their use of the term “stimulation”.

Activation refers to exposing naive T cells to inflammatory signals, while stimulation specifically refers to the T cell:antigen presenting cell interaction. “Priming” (activation) of naive T cells appears to be necessary before stimulating them with a mature antigen presenting cell. This was not a point made by any of the speakers in our video collection, but is an important consideration for designing experiments.