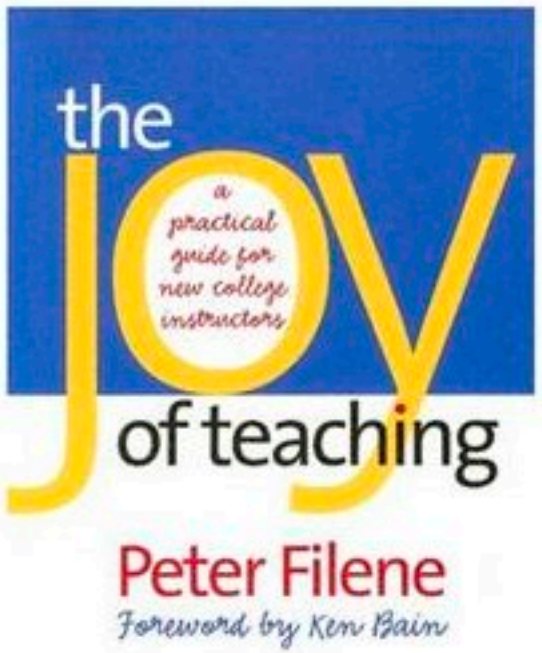
How we got here
What we do
You


the
joy
a practical guide for new college instructors
of teaching
Peter Filene
Foreword by Ken Bain

I bring to teaching a belief that_____

In the classroom I see myself as_____

I believe students are _____

I seek to foster in students_____

I think learning is_____

What do you want in life?

What do your students want in life?

open, empowering, and interactive

The big picture is:

Our interactions with microbes are fascinating and life-altering, and the study of these interaction brings healing and health. The skills I learn here can be applied to improve many areas of my life.

The main questions we are interested in are:

1. how microbes can be beneficial or detrimental and, in the latter case, how they cause disease
2. how the immune system defends us
3. how the scientific method brings knowledge, healing, and success to many aspects of our life even outside of science

This year's theme: VIRULENCE – HOW PATHOGENS ATTACK

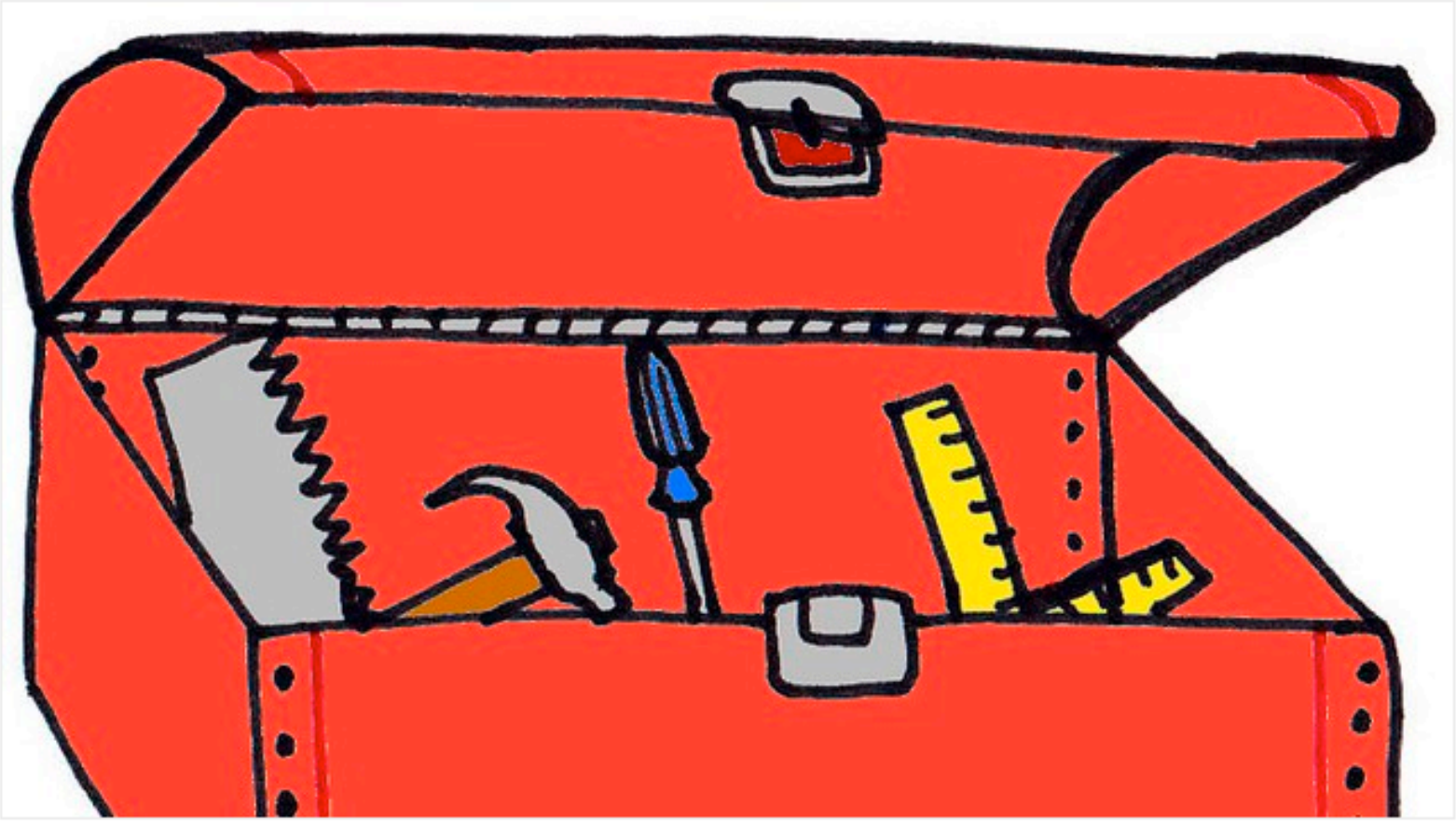
Outcomes – At the end of this class YOU will become more skillful in:

1. knowing how microbes protect us
2. knowing how microbes cause disease
3. knowing how the immune system protects us
4. knowing how the immune system causes disease
5. knowing how microbial disease is diagnosed and treated
6. taking charge of your own learning
7. reading and understanding primary literature; understanding the scientific method; knowing how the scientist thinks, problem solves, and performs research to benefit our lives. These skills will help you solve challenges in your professional and personal lives and learn new things to enrich your life.
8. being confident in tackling new questions and challenges

9. researching and communicating to others about science, disease, and health. YOU can be a light of knowledge to your family and friends in matters of life and death.

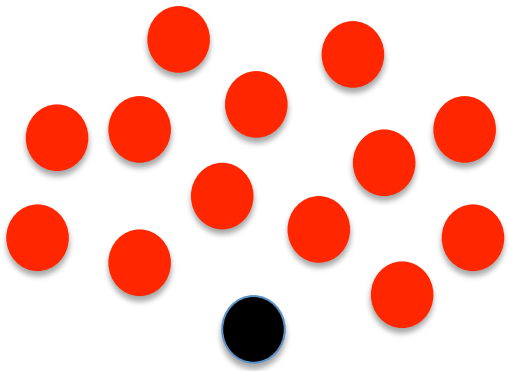
Scientific method

But you won't cover all the material!



Helping students learn how to learn: That's what most educators strive for, and that's the goal of inquiry learning. That skill transfers to other academic subject areas and even to the workplace where employers have consistently said that they want creative, innovative and adaptive thinkers. Inquiry learning is an integrated approach that includes kinds of

Complacent?

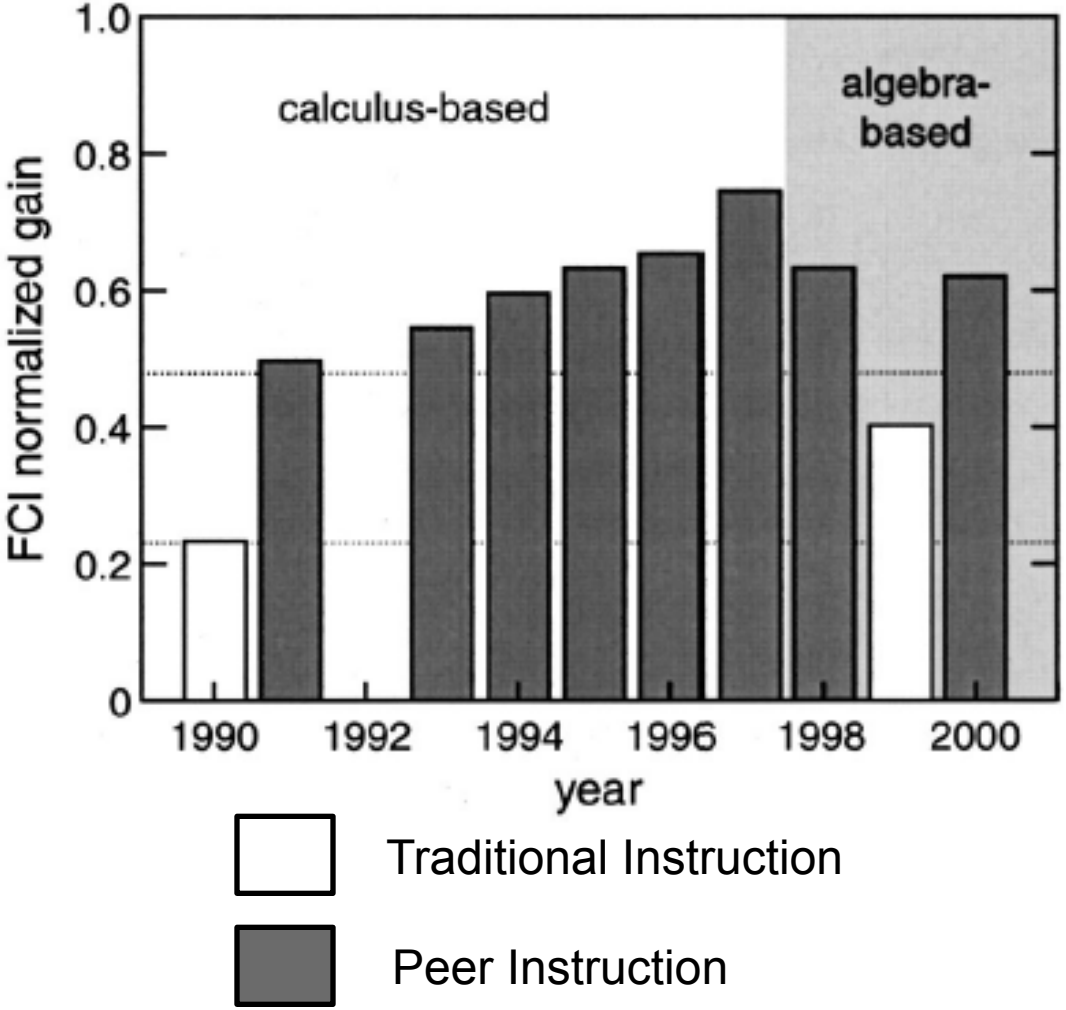


Lecture: Peer Instruction

- Are you prepared? (quick quiz at beginning of class, using clickers)
- Pose carefully designed question
  - Solo vote: Think for yourself and select answer
  - Discuss: Analyze problem in teams of 3-4 (set up in section)
    - Practice analyzing, talking about challenging concepts
    - Reach consensus
    - If you have questions, raise your hand and I or the TAs will come around
  - Group vote: Everyone in group votes
    - You must all vote the same to get your point
  - Class wide discussion:
    - Led by YOU (students) – tell us what you talked about in discussion that everyone should know!

Peer Instruction:
Learning Gains in Physics Nearly Double

Crouch, C., Mazur, E. Peer Instruction: Ten years of experience and results



Clickers vs. Peer Instruction

- Discussion with your peers is KEY!
  - Testing yourself can help you know if you know it
  - But LEARNING happens during the discussion!

Pre-Class preparation
Quiz/Incentive/Feedback

1) Individual Thinking, Vote
2) Group Discussion (with 1-2 other students)
3) Group Vote

Class-wide discussion
Student-led/Instructor Modeling/Mini-lecture

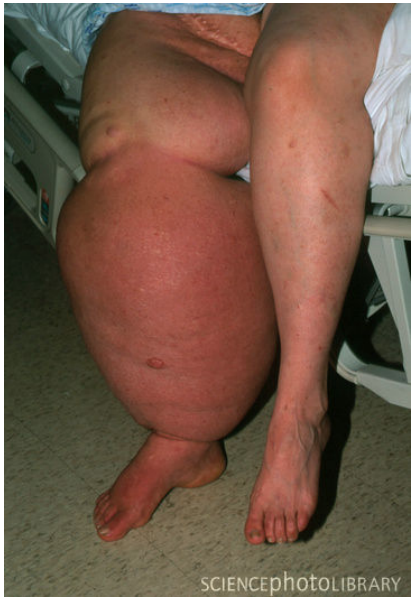
Reading comprehension: which of these is not true of antibodies:

A. can neutralize toxins
B. can promote phagocytosis
C. can induce cell killin
D. can trigger complement activation
E. can stimulate neutrophils to multiply

Reading comprehension: These T cells fight autoimmunity and curtail excessive effector immune activity

A. TH1
B. TH2
C. Treg
D. TH17
E. All of the above

Brugia malayi is a roundworm (nematode) parasite that causes serious disease in humans (see picture). Scientists isolated PBMCs (Peripheral Blood Mononuclear Cells; T cells, B cells, monocytes) from people's blood and exposed them to BmA (a highly antigenic protein made by the worm) and then measured the release of various cytokines. If you took PBMCs from someone in the US where there is no *B. malayi* and someone in India where there is a lot, what might you expect to see?



A. PBMCs from both patients treated with BmA will produce IFN-γ and/or other cytokines
B. PBMCs from neither patient treated with BmA will produce IFN-γ and/or other cytokines
C. PBMCs from the American patient (but not the Indian patient) treated with BmA will produce IFN-γ and/or other cytokines
D. PBMCs from the Indian patient (but not the American patient) treated with BmA will produce IFN-γ and/or other cytokines
E. PBMCs from both patients will produce IFN-γ and/or other cytokines without treatment with BmA

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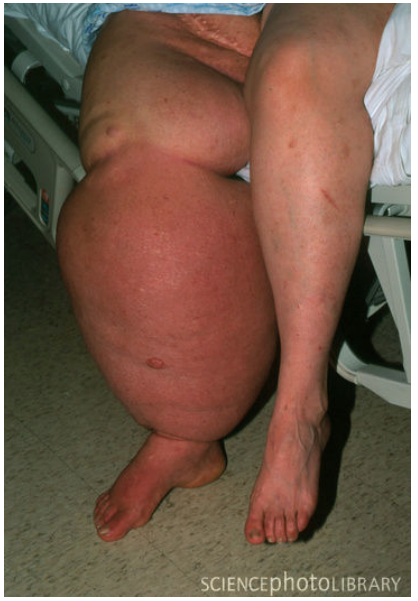

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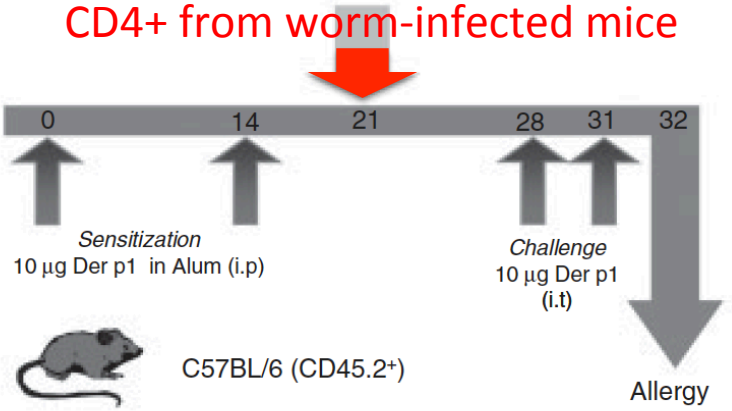
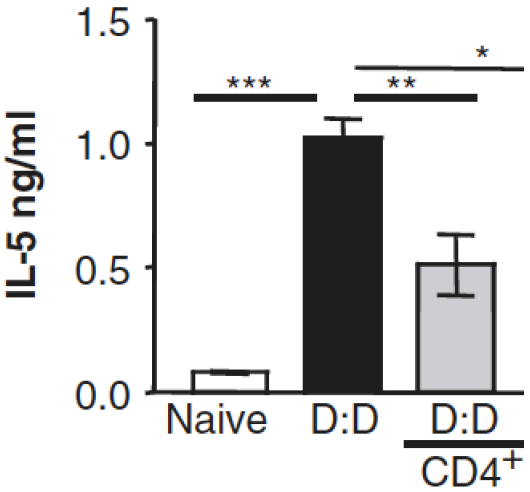
D. PBMCs from the Indian patient (but not the American patient) treated with BmA will produce IFN-γ and/or other cytokines
E. PBMCs from both patients will produce IFN-γ and/or other cytokines without treatment with BmA

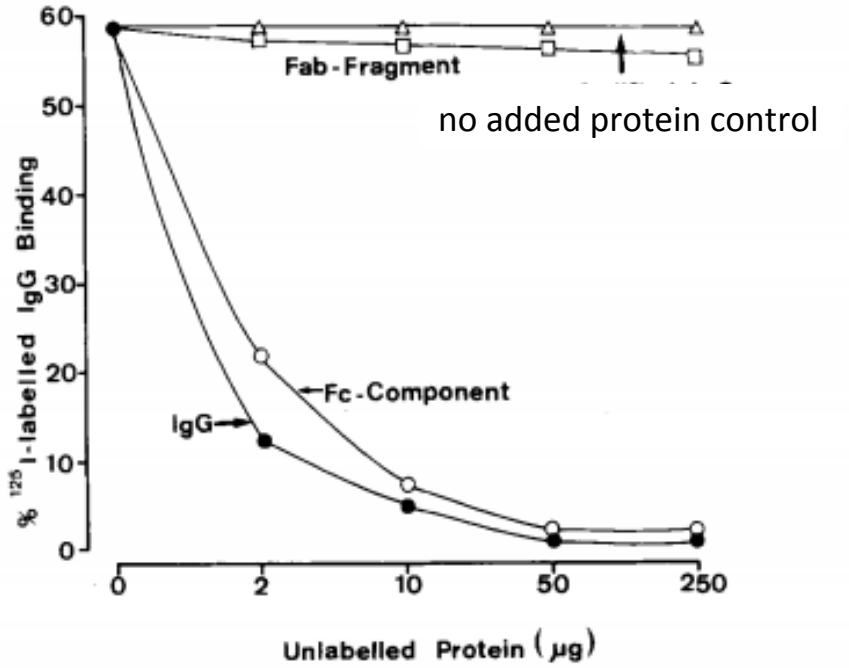
In the figure upper right, mice were sensitized twice on day 0 and day 14 with Der p1 (dust mite protein) along with an adjuvant to induce an immune reaction. On day 28 and 31, the mice received Der P1 intratracheally (challenge), which resulted in airway allergy. The lungs were then washed with fluid and the amount of IL-5 present in the lung fluid measured (black D:D bar in figure below; IL-5 is a cytokine secreted by T cells that is thought to play a major role in strong allergic disease such as asthma). Naïve means IL-5 levels in lung fluids of mice not sensitized to Der P1 on days 0 and 14. In addition, in one set of experiments, on day 21 between sensitization and allergy induction, the mice were injected intravenously with CD4+ cells from the lymph nodes of mice infected with parasitic worms (red). IL-5 levels were measured again (gray bar D:D/CD4+). What is going on?

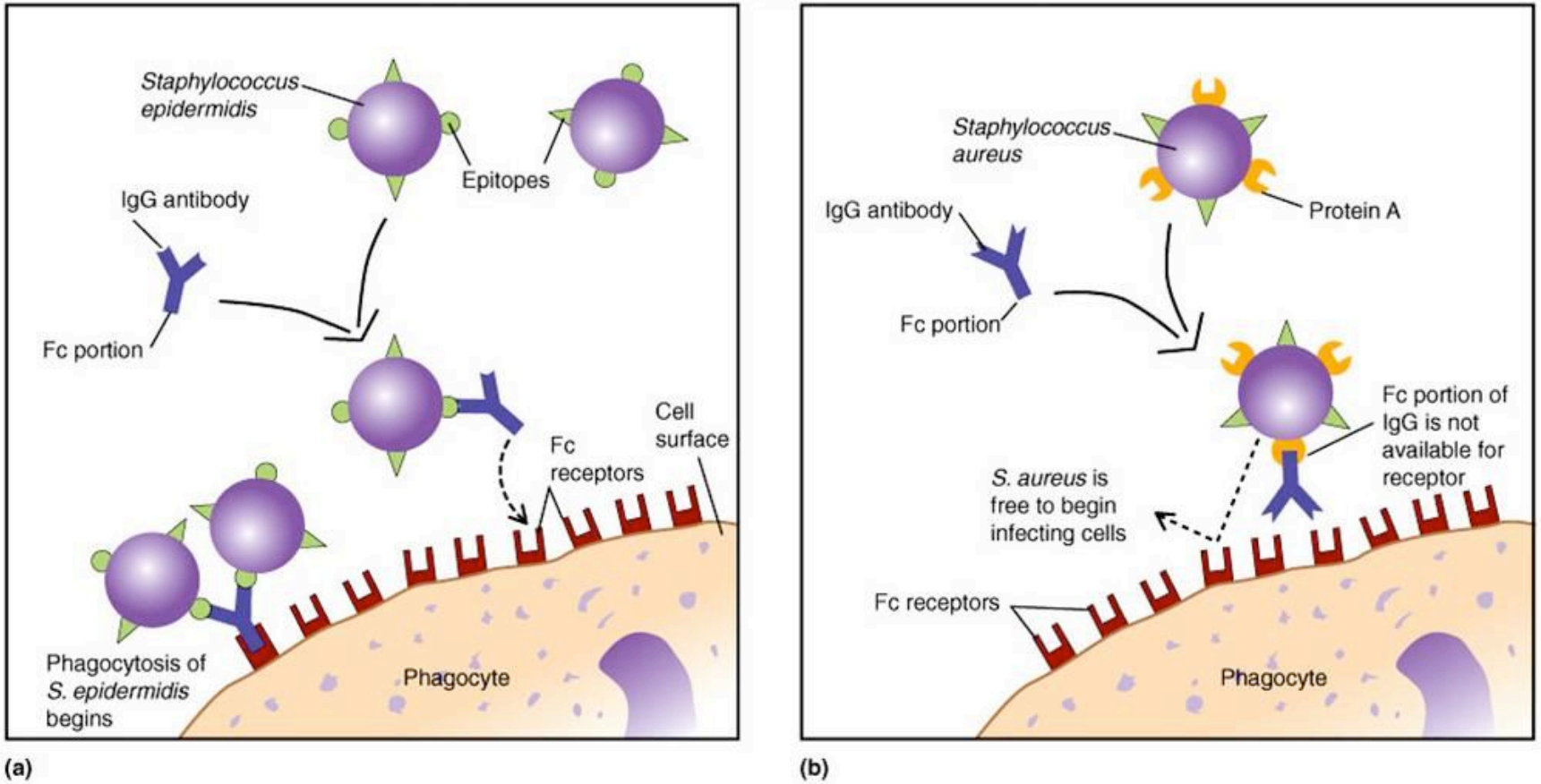
ALWAYS FEEL FREE TO RAISE YOUR HAND IF YOU HAVE A QUESTION





The following is an interesting experiment. Intact *Streptococcus* bacteria were incubated with radio-active labeled intact total IgG antibody (0.2 μg) isolated from naïve (uninfected) humans. The bacteria were then sedimented by centrifugation and washed to remove all non-bound antibodies. High binding of IgG, as detected by high radioactive, was seen (i.e., 60% of the IgG was bound when 0 μg of unlabelled protein was added). The experiment was then repeated in the presence of increasing amounts of various unlabeled proteins: total IgG, Fc portion of antibodies, Fab fragment of antibodies, or nothing (no added protein control). What is the bacteria doing and to what end? Raise your hand if you are lost and/or eventually need a hint.

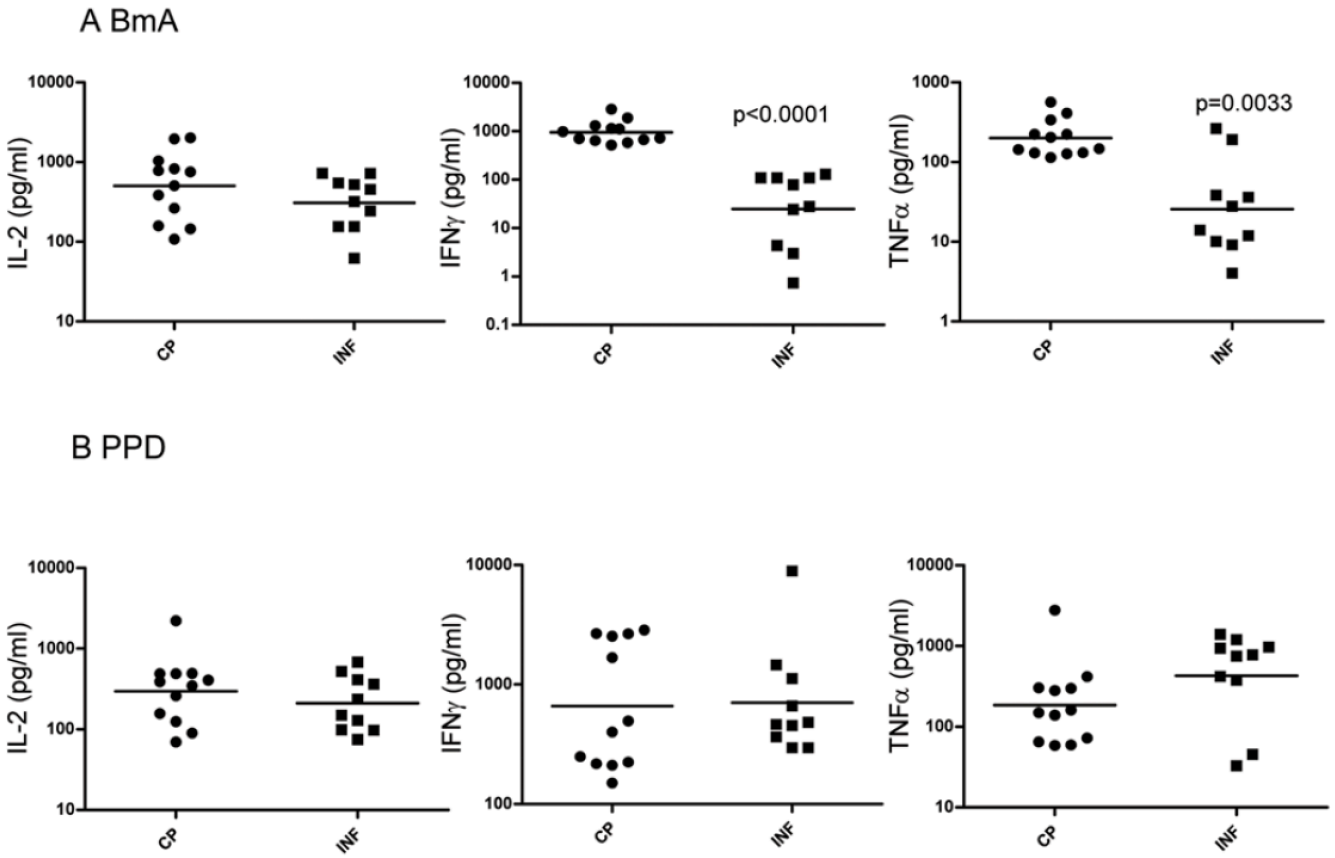




Researchers took PBMCs from Indian patients who had swollen legs (CP) and from Indian patients who had worms but no swollen legs (INF) and measured cytokine levels (each dot is measurements from one person). Here are there results (BmA is Brugia antigen; PPD is Mycobacterial antigen):

How do you interpret these results?

1. People with symptoms have higher TH1 response
2. People with symptoms have higher TH2 response
3. People with symptoms have lower TH1 response



4. People with symptoms have have lower Th2 response

5.People with Brugia infections are more susceptible to tuberculosis.

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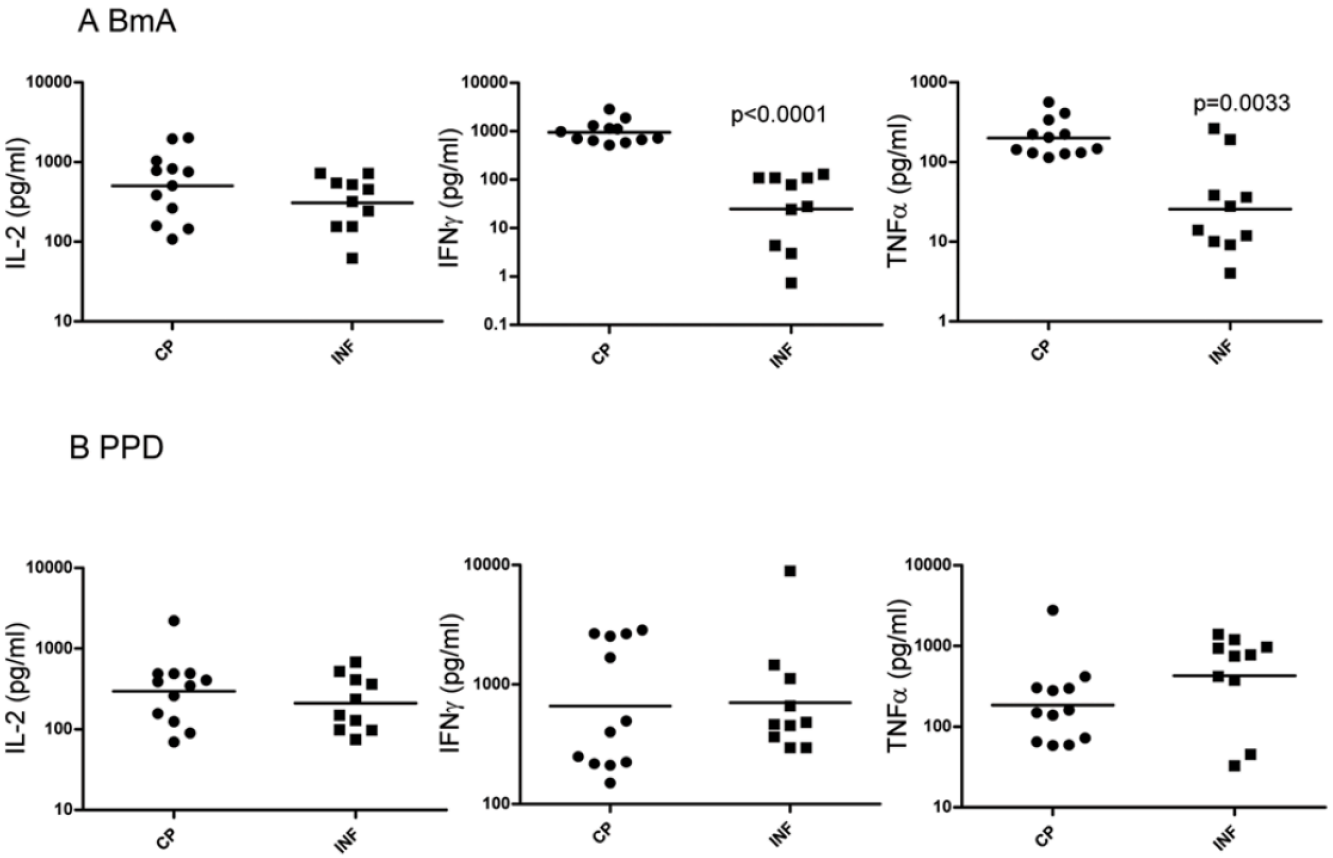

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5.People with Brugia infections are more susceptible to tuberculosis.

Charles Janeway, a famous immunologist, was noted for calling out other immunologists for a "dirty little secret". This secret involves a surprising result seen when an antigen made of *pure foreign protein* like ovalbumin was injected into an animal (e.g., mouse) to induce an immune response. What was the surprising result?

1. The injection of pure protein resulted in a massive TH1 inflammatory response that killed the animal

2. The injection of the pure protein resulted in no immune response and tolerance to the antigen

3. The injection of the pure protein resulted in over-production of antibody to the protein, which then killed self-cells by ADCC

4. The injection of the pure protein made the animal generally more susceptible to bacterial and viral infections

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What was missing in the pure protein experiment such that no antibody response was seen?

1. A signal from complement, which pure protein cannot activate, required to trigger the adaptive immune system

2. Stimulation of pattern recognition (toll-like) receptors, signaling that a pathogen was present

3. A lipid component to mimic the presence of a pathogenic bacterium

4. A secondary boost of protein, required to trigger a robust immune and memory response

5. A tissue wound or physical damage to alarm the animal that a pathogenic attack was actually present

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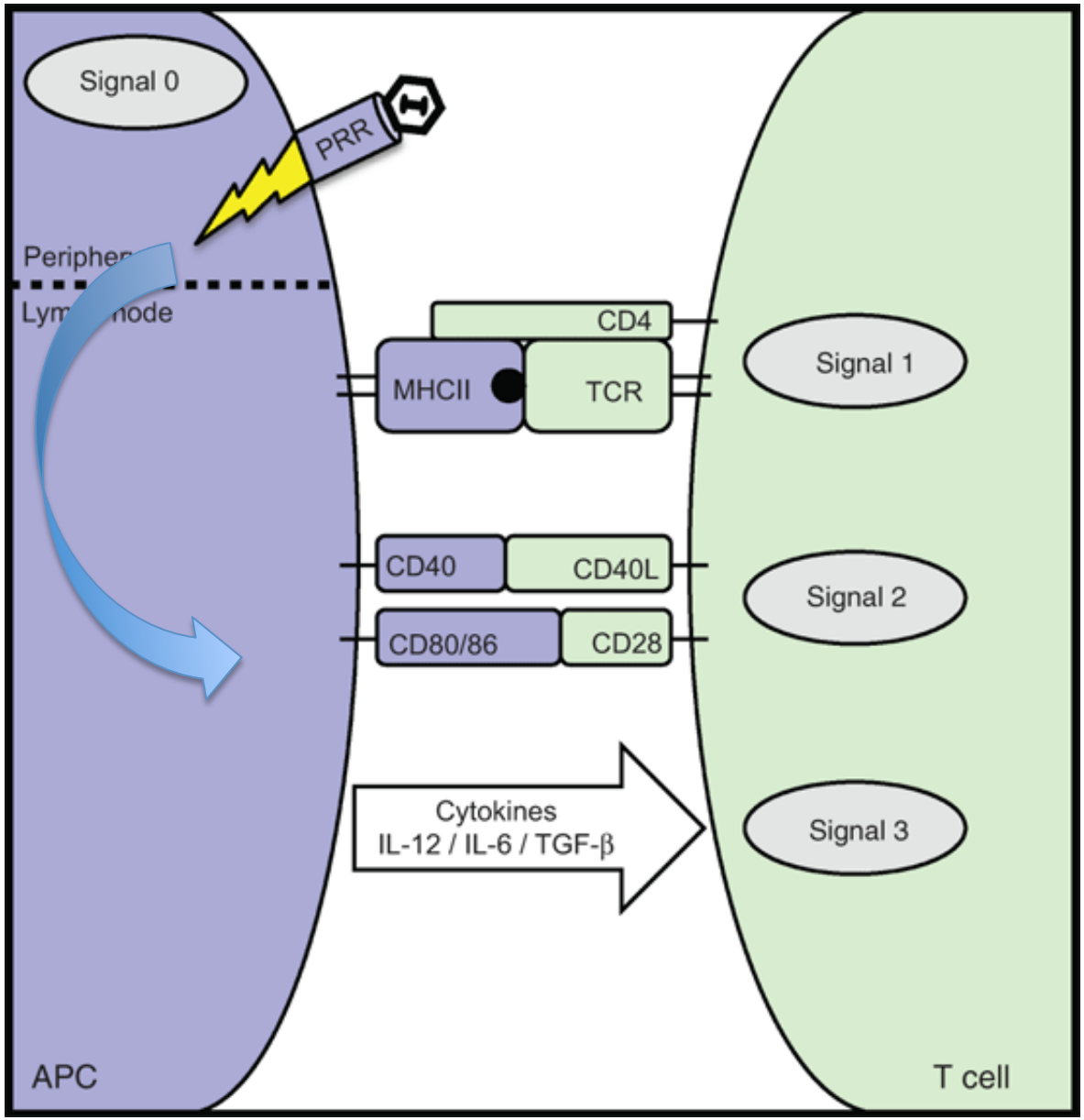
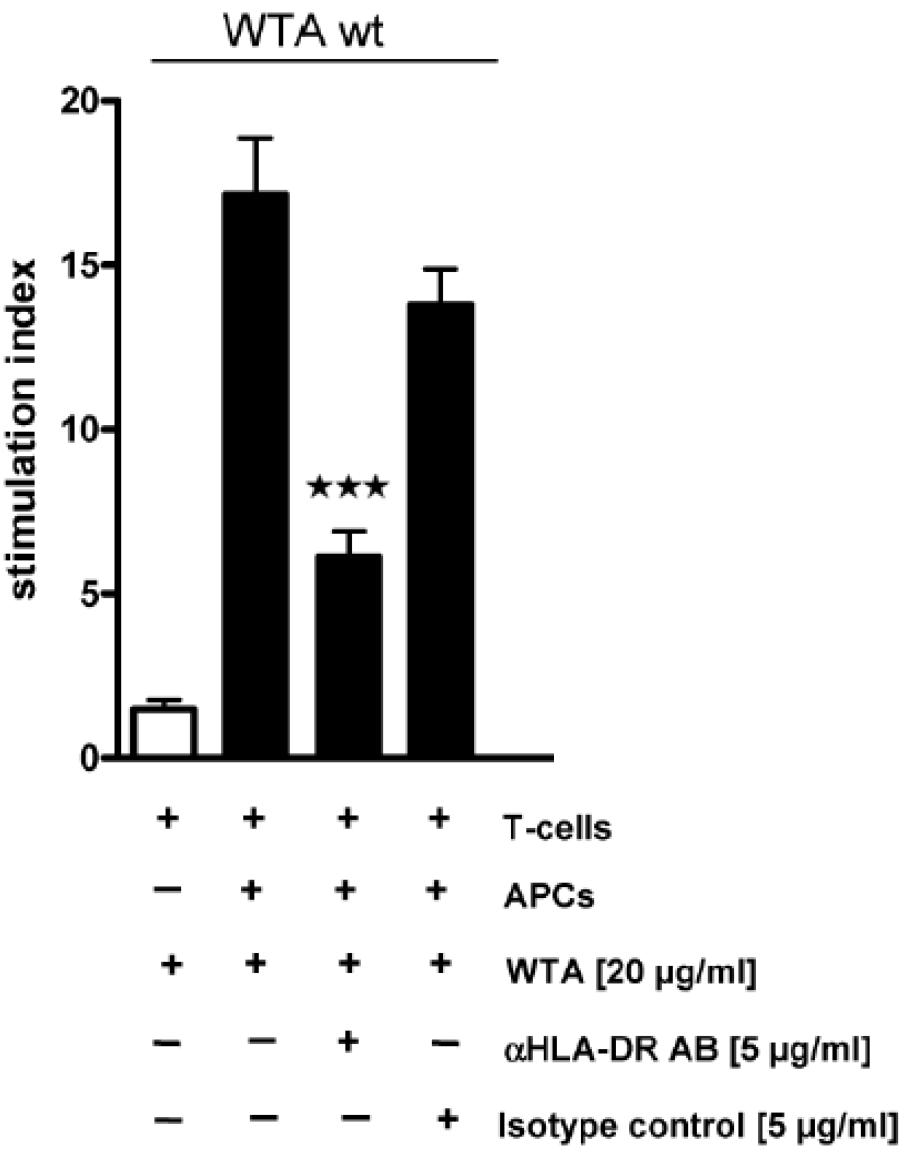

Signal 0
PRR
Peripher
Lym node
CD4
MHCII
TCR
Signal 1
CD40
CD40L
Signal 2
CD80/86
CD28
Cytokines
IL-12 / IL-6 / TGF-β
Signal 3
APC
T cell

In the experiment to the right, naïve T cells were isolated from mice as were naïve antigen presenting cells (APCs; naïve means that the mice had never been exposed to pathogen). The cells were mixed together along with WTA (*Staphylococcus aureus* cell Wall Teichoic Acid). In some cases, an neutralizing antibody to MHC II was added (αHLA-DR AB). The researchers then measured how stimulated the T cells were (actually measured how much they divided).

What is WTA doing to the cells? Why do you think S. aureus might do this?



In the figure below, HEK (human kidney cells) were transformed with DNA to express TLR1 and TLR2 receptors. They were then treated with various amounts of Pam3cys (x-axis), which mimics bacterial lipoprotein along with various amounts of a protein called SSL3. They then measured the amount of a pro-inflammatory mediator called IL-8 produced by these cells. Lipoteichoic acid was shown to behave the same as Pam3cys in this experiment. What is your best guess about who makes SSL3 and to what purpose?

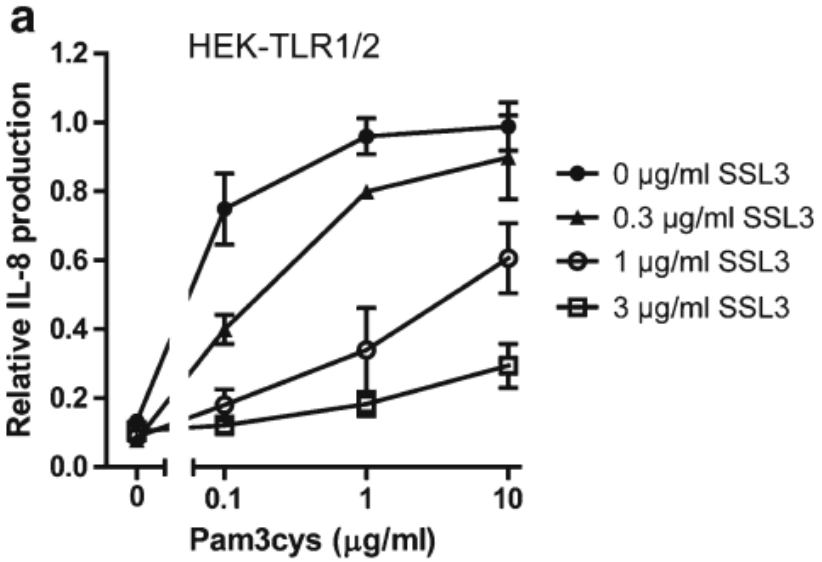
A. Made by a gram-negative bacterium to induce inflammation

B. Made by a gram-negative bacterium to inhibit the immune system

C. Made by a gram-positive bacterium to induce inflammation

D. Made by a gram-positive bacterium to inhibit the immune system

E. Made by the immune system to up-regulate immune responses



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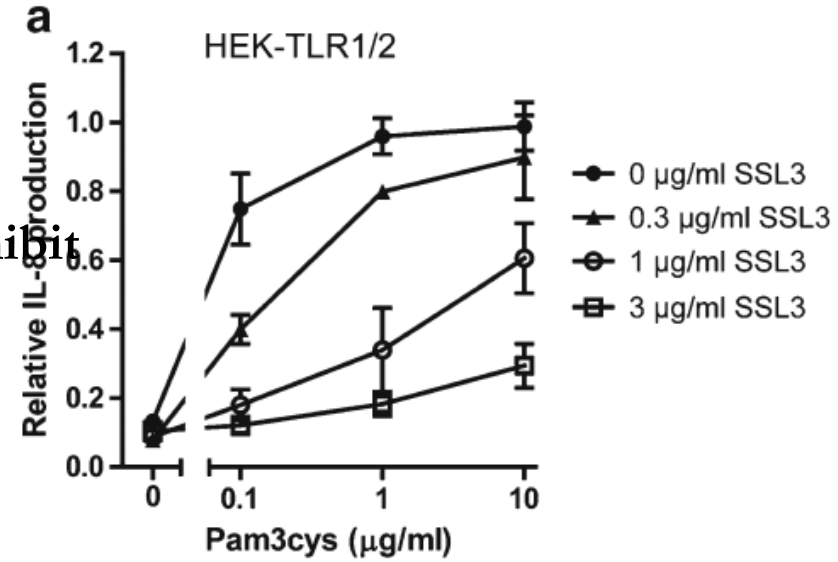
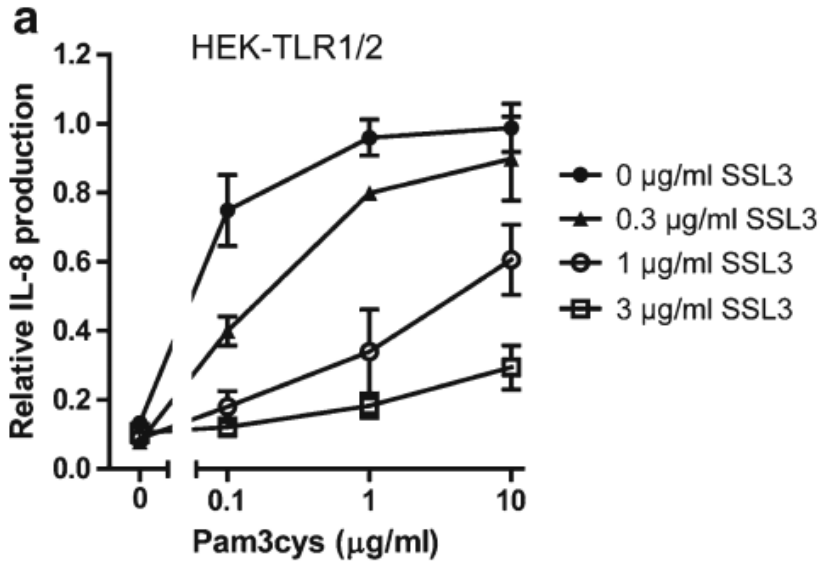


Our theme for the class: SSL3 is a bacterial **virulence factor** made by *Staphylococcus aureus*



Virulence factors are molecules expressed and secreted by pathogens (bacteria, viruses, fungi and protozoa) that enable them to achieve the following:

- colonization of a niche in the host (this includes adhesion to cells) for example, Trimeric Autotransporter Adhesins (TAA)
- Immunoevasion, evasion of the host's immune response
- Immunosuppression, inhibition of the host's immune response
- entry into and exit out of cells (if the pathogen is an intracellular one)
- obtain nutrition from the host.

If the experiment were repeated with HEK cells *without* DNA to express TLR1/2 (so no TLRs in the cell at all), would you expect the resulting response to increasing Pam3cys to look more like the line for:

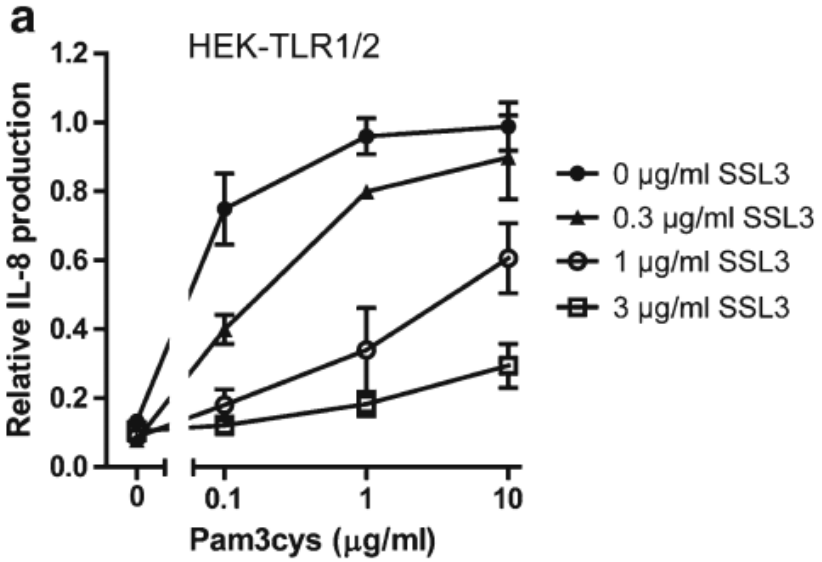
A. 0 μg/mL SSL3

B. 0.3 μg/mL SSL3

C. 1.0 μg/mL SSL3

D. 3.0 μg/mL SSL3

E. Can't be determined



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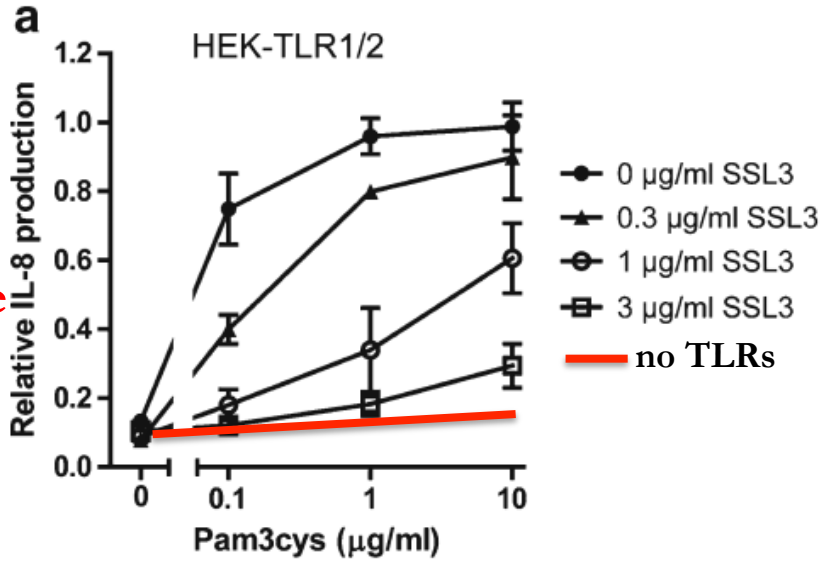
E. Can't be determined

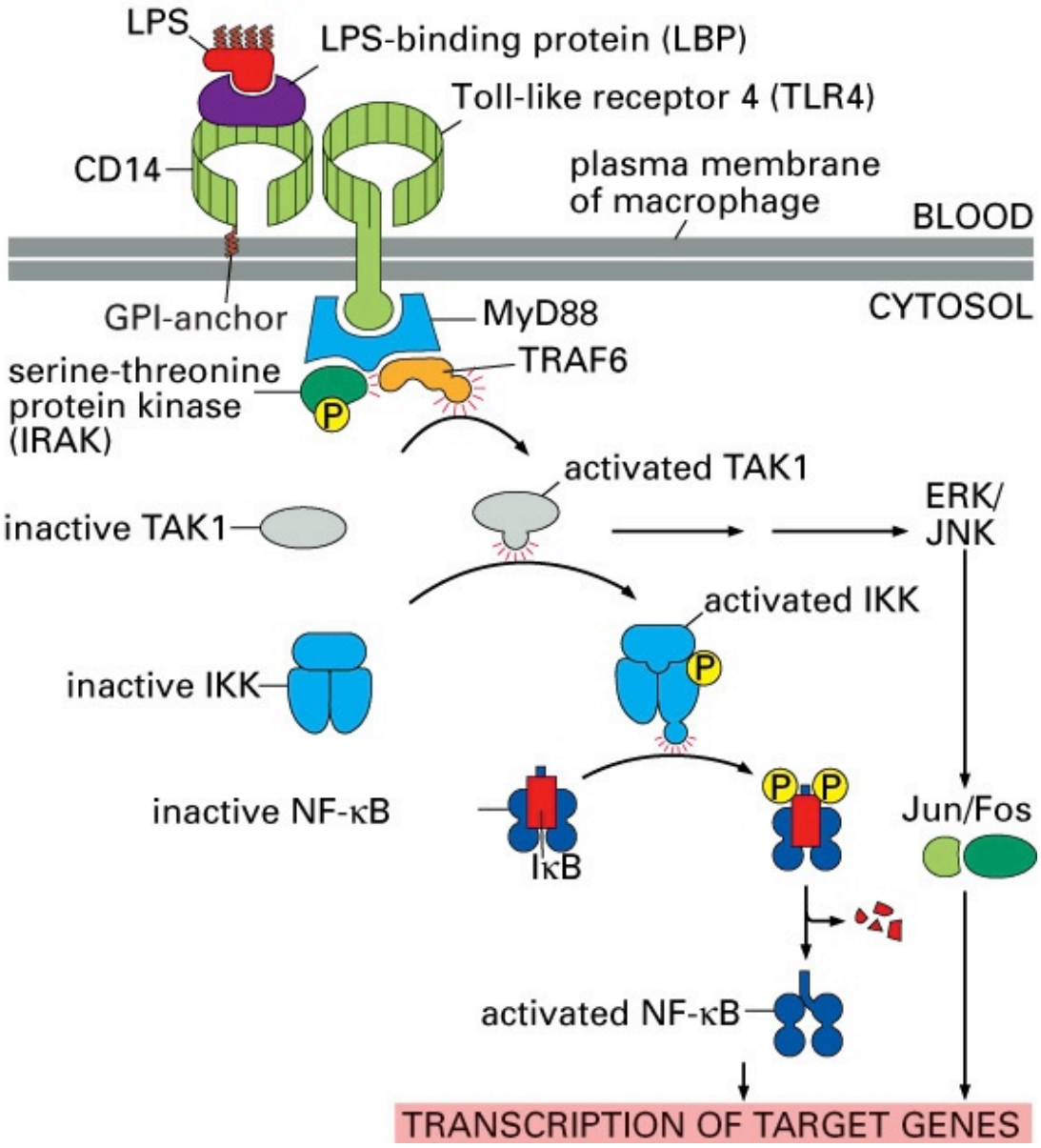
AND THIS IS AN IMPORTANT CONTROL

an experimental condition included to eliminate alternative or trivial explanations of the results and allow focus on one variable at a time.



Induction of Cytokines

MAPKKK



In this experiment, the bacterium Staphylococcus aureus was incubated with serum (which contains all the complement components) and either without (w/o) or with (+) the bacterial protein called Sbi for 20 minutes. Then the bacteria were taken away from the serum and Sbi (so no serum and Sbi were left) and then incubated with human macrophages (hint: macrophages remove bacteria via opsonization). Negative control is a tube with just macrophages and no bacteria. After the time indicated, the bacteria were recovered from the medium and the number of live bacteria counted (CFU). Which of the following statements is most consistent with the result? (bonus– what is the purpose of the negative control)

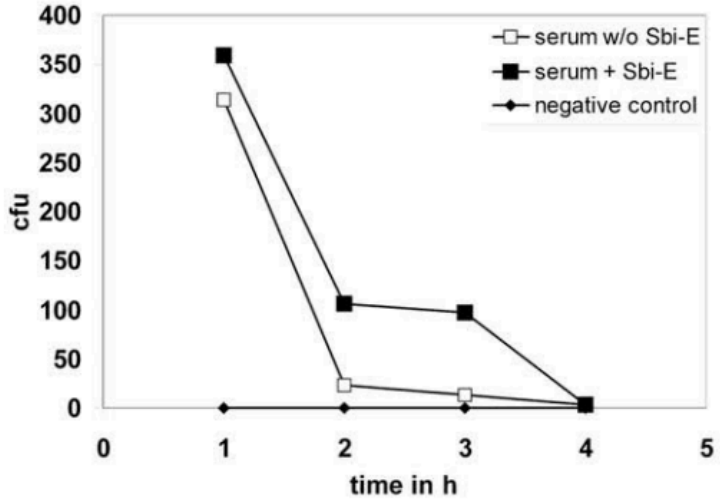
A. The Sbi protein inhibits the deposition of C3b on the bacterial surface.

B. The Sbi protein enhances the deposition of C3b on the bacterial surface.

C. The Sbi protein eventually kills all macrophages.

D. The Sbi protein activates macrophages.

E. Sbi inhibits activation of TLRs.



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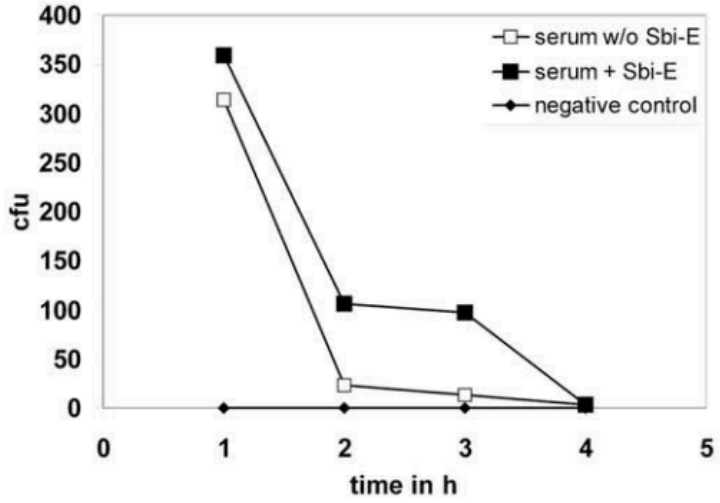


In the same paper, it was shown that Sbi binds to and promotes the recruitment of factor H to the bacterial surface (see pg. 78, Table 6-6). Is this consistent with what we just saw? Is Sbi a virulence factor?

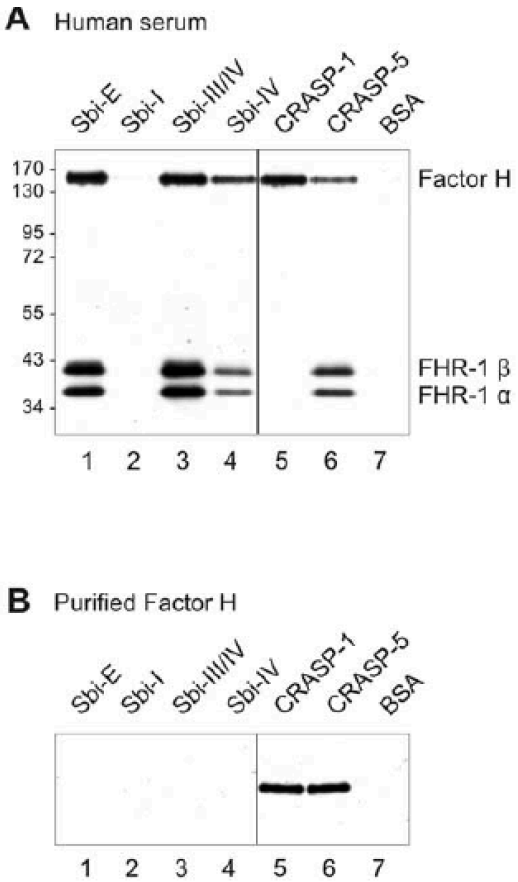
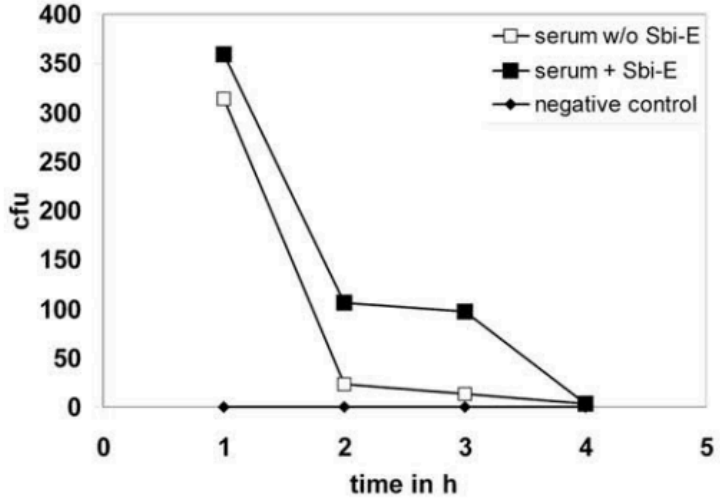
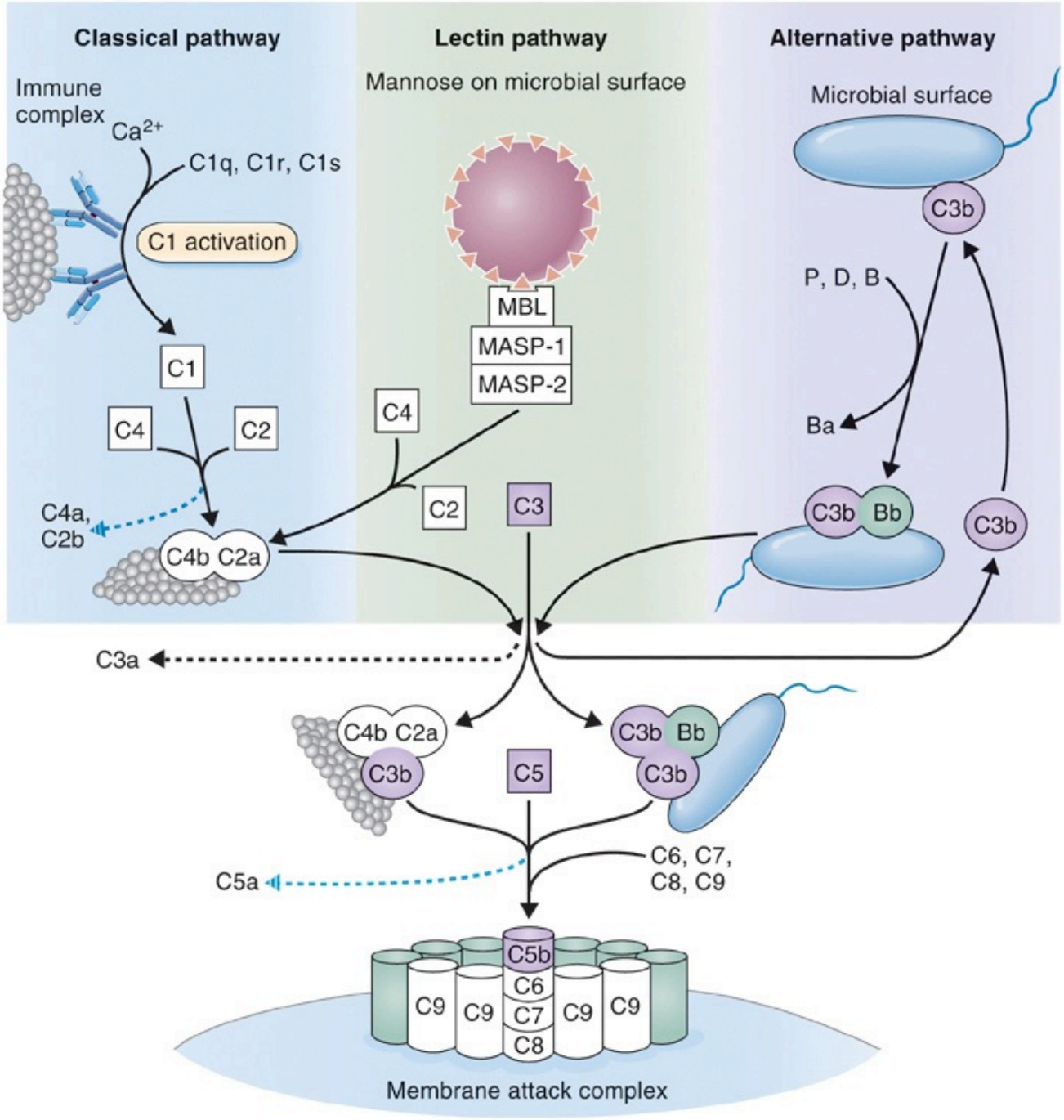
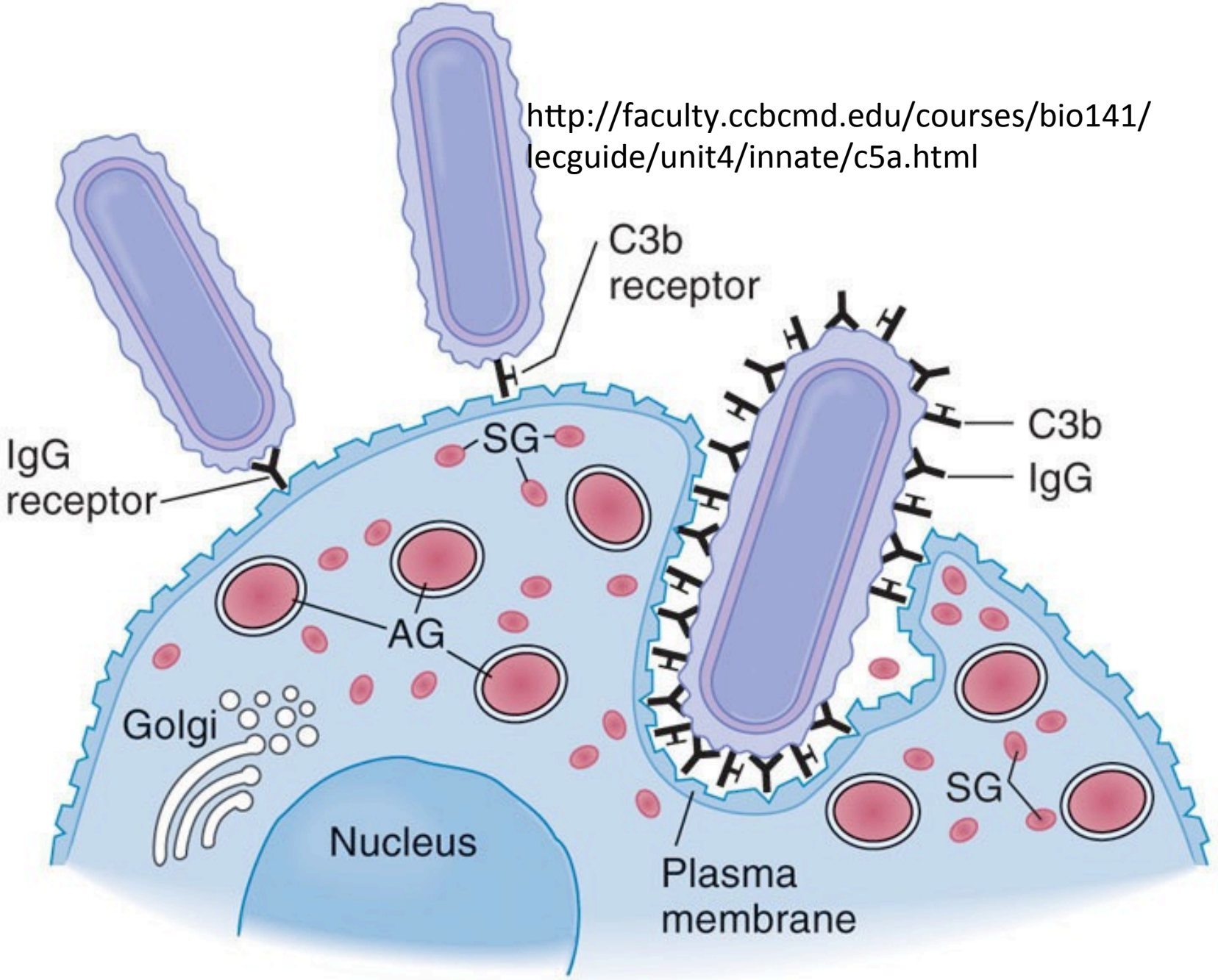


Figure 3. Binding of Factor H and FHR-1 to Sbi. (A) CEWA was used to analyze interaction of host complement regulators with the IgG binding Sbi protein. Sbi-E, Sbi-I, Sbi-III/IV and Sbi-IV were immobilized onto the surface of a microtiter plate and human serum was applied. After extensive washing bound proteins were eluted, separated by SDS-PAGE and identified by Western blotting based on their mobility and their reactivity with mAB C18 that is specific for the C-terminal SCR domain of Factor H and FHR-1. The borrelial Factor H binding CRASP-1, the Factor H/FHR-1 binding CRASP-5 and BSA were used as controls.

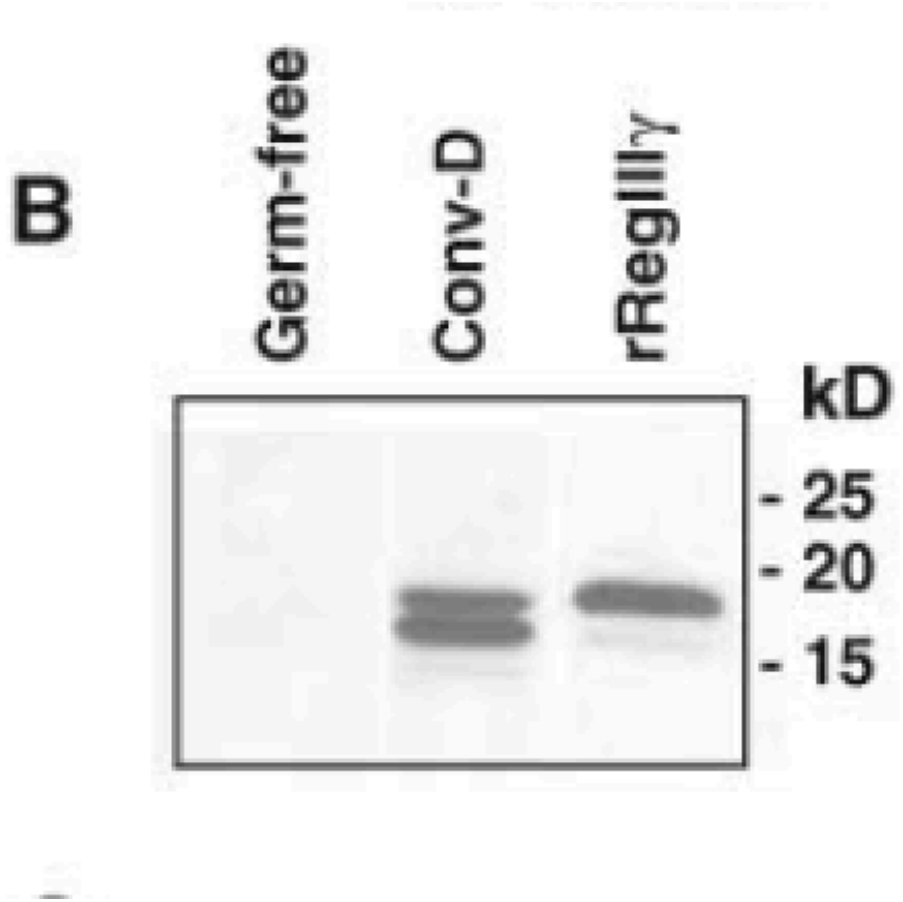


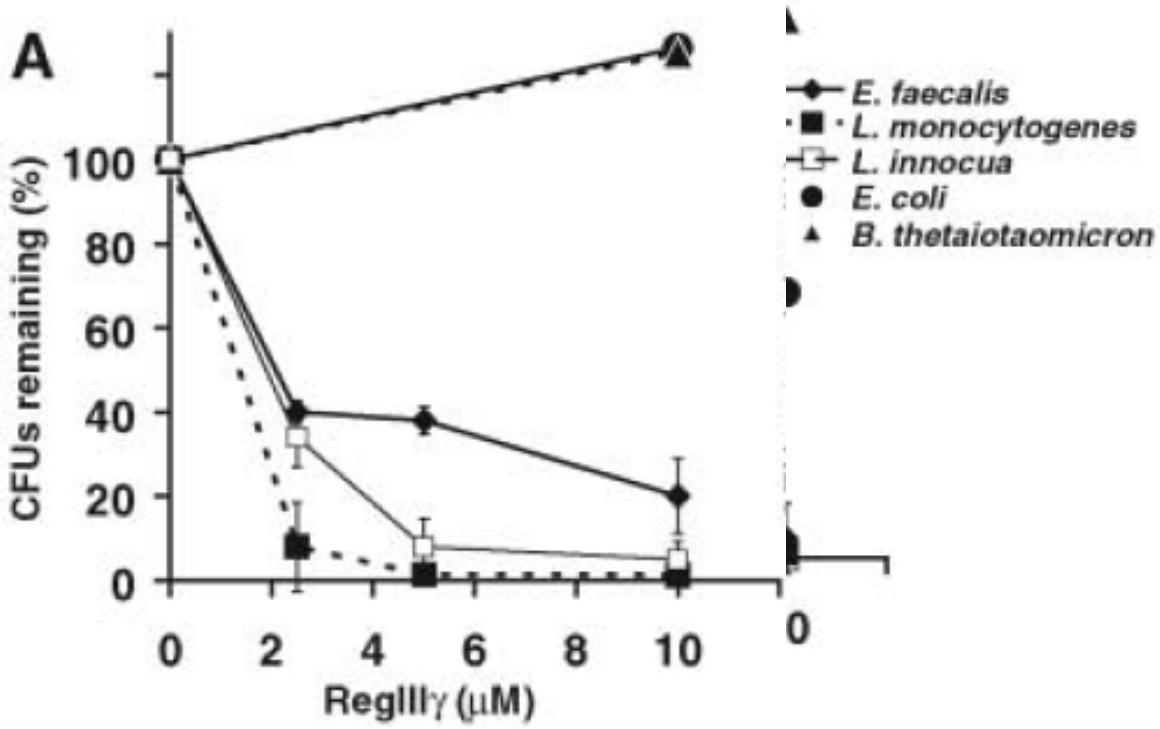


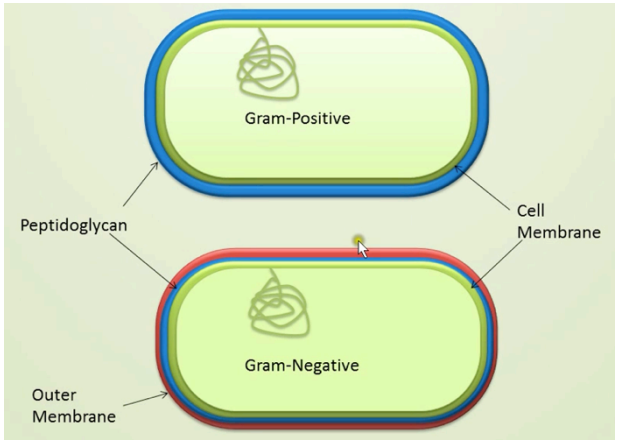
http://faculty.ccbcmd.edu/courses/bio141/lecguide/unit4/innate/c5a.html



Antimicrobial peptide: part of the innate immune system. Made by macrophages, neutrophils, and epithelial cells (induced by TLRs) these peptides play an important role in immunity. In this experiment, researchers took mice that lacked intestinal bacteria (germ-free) and then added bacteria back (conventionalized or Conv-D). They looked at various proteins made by the intestine and saw this when they blotted with antibodies to a small protein called RegIIIγ. What is the result?

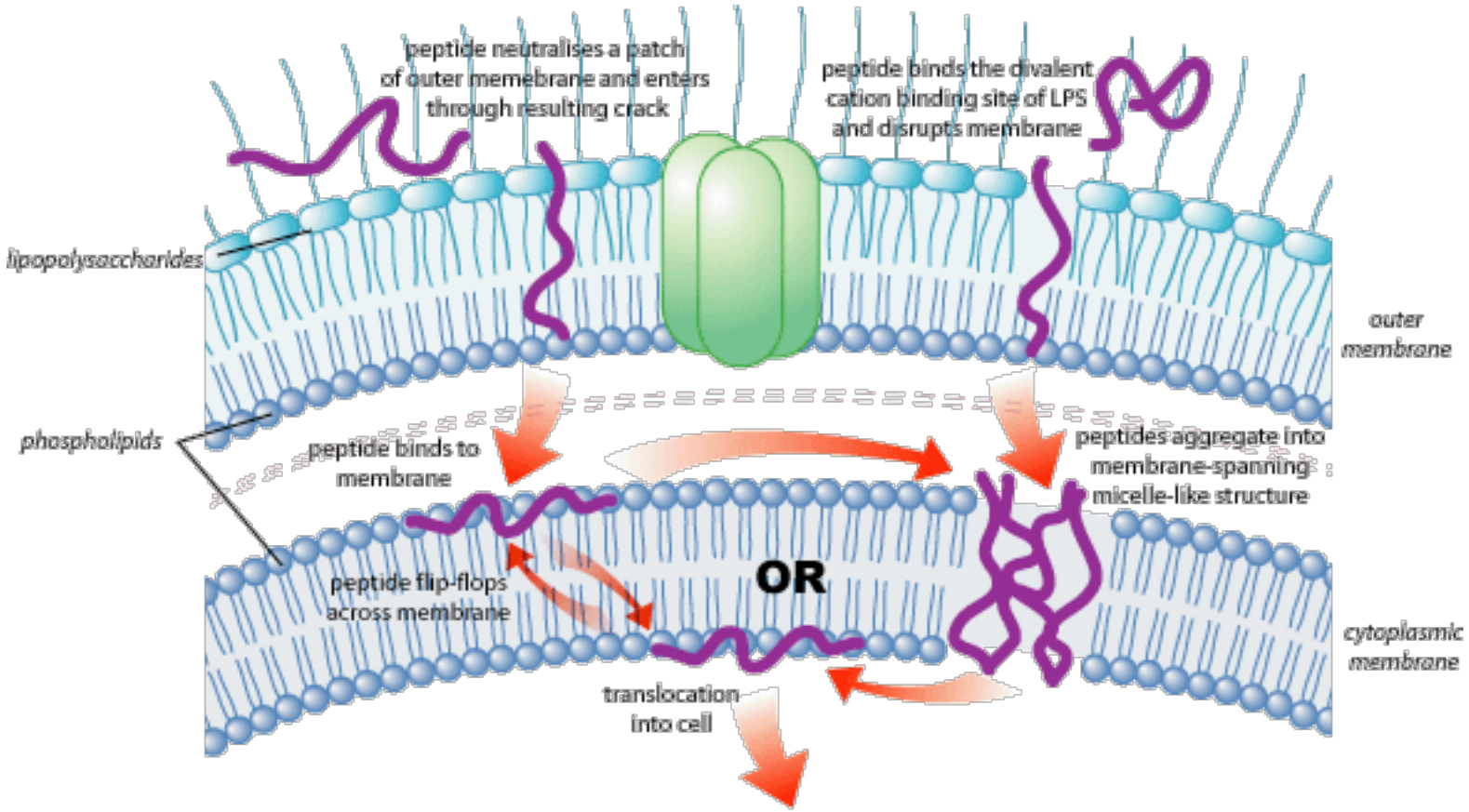






This Figure from Cash et al., Science, 2006, shows killing curves of a peptide RegIIIγ secreted by intestinal cells when they come in contact with bacteria. Lower CfUs means more killing of bacteria. E. coli and B. thetaiotamicron are Gram negative bacteria. E. faecalis, L. monocytogenes, and L. innocua are Gram positive bacteria.

What part of the bacterial cell wall might RegIIIγ be binding to? A. LPS, B. Porin, C. Plasma membrane, D. Peptidoglycan, E. Lipoteichoic acid?


peptide neutralises a patch of outer memebrane and enters through resulting crack
peptide binds the divalent cation binding site of LPS and disrupts membrane
lipopolysaccharides
outer membrane
phospholipids
peptide binds to membrane
peptides aggregate into membrane-spanning micelle-like structure
peptide flip-flops across membrane
OR
translocation into cell
cytoplasmic membrane

Complement:

- humoral defense (proteins found in blood)
- needs to be activated
- Many functions:
  - 1. recognizes pathogens but differently than TLRs
  - 2. does something about the pathogens
    - Causes them to be more "sticky" so immune cells can gobble them up
    - Lyses some bacteria and viruses directly
    - Attracts killer white blood cells to the source of bacterial infection
    - Loosens up blood vessels so that white blood cells can crawl to point of bacterial infection
    - These activities by their very nature promote inflammation
    - Activates other immune cells including antimicrobial systems of phagocytes, antibody responses of B cells, T cells responses
- Should be obvious that over-reaction of complement is very bad for the body; hence tightly regulated

complement

http://faculty.ccbcmd.edu/courses/bio141/lecguide/unit4/innate/c5a.html

http://www.microbiologytext.com/index.php?module=Book&func=displayfigure&book_id=4&fig_number=9&chap_number=15