HOST DEFENSE

SMALL GROUP PROBLEM SOLVING SESSION

LYMPHOCYTE CYTOTOXICITY
Small Group Classrooms

LEARNING GOAL
You will be able to appreciate the critical importance of T-cell and natural killer cell cytotoxicity.

OBJECTIVES
• Discuss T-cell and NK cell mechanisms that control viral infection.
• Identify host situations that attenuate T-cell cytotoxic responses.
• Explain the clinical presentation of viral infections in patients with deficient T-cell responses.
• List the clinical implications of T-cell cytotoxicity.

BACKGROUND READING

DEVELOPED BY
John A. Robinson, MD
Before coming to class:

1. Read assigned chapters/pages and develop answers for **ALL** the questions in the 4 clinical vignettes

During the Small Group Session:

2. Each small group (should be 4-5 peers- please do not sort yourselves into large groups-you will learn much less) should discuss the four case studies and decide the best solutions to the specific integrating questions associated with each case.

3. After approximately an hour of discussion by the subgroups, the facilitator will recapitulate the answers to the integrating questions by selecting a subgroup to present a synthesis of their relevant discussions to the entire group. Facilitators will select, at their discretion, a small group for the discussion of the individual cases.

4. History has shown that students who don’t contribute to the Small Groups do not do well in the Course (remember that about 25-30% of the final comes from small groups!) and also have been assaulted by their fellow group members.

5. At the end of the session, a master answer sheet will be posted on the Host Defense website.

**CASE 1**

**LYMPHOCYTE CYTOTOXICITY**

A 38 year old female who had 7 children by 3 different fathers developed a dilated cardiomyopathy and severe heart failure. She required a cardiac transplant. Fourteen days after the transplant an endomyocardial biopsy of the right ventricle was done to assess whether the patient was beginning to develop rejection in the allograft. The biopsy revealed the presence of multiple types of graft-infiltrating cells.

1.
   - Predict the phenotypes of the cells found in the biopsy when analyzed by classical histology.
   - How might **new** methods increase or change the “classic” findings?
   - What are the mechanisms that attracted immune effector cells into the graft?

2.
   - Discuss the probabilities that this patient may continue to have significant episodes. The key point to consider is that she has multiple children by 3 men and let’s assume the fathers were not identical triplets.

Rev 11/29/2011
• What in vitro tests could be done that would have predicted rejection?

3. If rejection becomes significant, design some monoclonal antibodies and/or other strategies that might be helpful in its treatment? Design a few that, in theory, increase tolerance to the transplanted organ. Speculate on what you could look for in surveillance biopsies to suggest you have succeeded.

4. Despite aggressive attempts to suppress rejection of the transplanted heart, the patient develops biventricular failure. Technical problems preclude the use of a ventricular support device. A xenotransplant is considered.

   • Select the optimal xenodonor and discuss the barriers to success.

CASE 2
LYMPHOCYTE CYTOTOXICITY

A 26 year old patient with acute leukemia undergoes intensive chemotherapy and then returns home. Shortly thereafter, he was visited by his 4 year old niece. The next day she becomes irritable and notices small vesicles (small, clear fluid containing blisters) on her arms, face and trunk that last about a week. Just as they were resolving, her uncle rapidly develops a fever, a diffuse hemorrhagic rash and then severe shortness of breath.

1. • Why was the unfortunate timing of the niece visit lethal for the patient?

2. • If a virus infected a cutaneous cell in a normal host, how would the immune system keep it localized and prevent spreading to neighboring cutaneous cells?

   • Describe the sequence of a normal response and contrast them to what happened in this patient?

3. The niece had a typical course of this type of viral infection - well circumscribed vesicles (tiny skin “blisters”) that resolved in 7-10 days - why did the uncle develop a completely different reaction? What would have been the clinical manifestations in the niece if she had been vaccinated previously?

4. How could it be treated? (Hint: a basic concept you learned in an earlier small group)

Case 3
Rev 11/29/2011
LYMPHOCYTE CYTOTOXICITY

A 21 year old male with cystic fibrosis receives 2 lungs from an unrelated cadaver donor and, in order to prevent rejection of the allograft, is treated with a medley of immunosuppressive therapies that inhibit T-cell function. The donor, but not the recipient, had been previously infected with a virus that causes infectious mononucleosis. About 8 weeks after transplantation, the patient develops rapidly enlarging lymph nodes, an enlarged spleen and severe symptoms of a sore throat coupled with high fevers and weight loss. The lymphocytes in his peripheral blood looked possibly malignant. By another amazing coincidence, the recipient has an identical twin and the recipient’s physician became aware of this fact.

QUESTIONS.
1. What childhood disease passed the patient by?
   Speculate on why he developed such a serious illness from a virus that usually produces mild disease.
2. Resistance to viral infection requires a coordinated host defense effort.
   Identify and discuss the most likely steps in this patient’s T-cell response that were rendered defective.
3. The Art of Medicine is based upon ingenuity and adapting to unique circumstances.
   Develop a novel therapy for this patient that might effectively treat his potentially malignant disease.
   Discuss, step by step, how the therapy would have to be developed.
4. What if a different recipient underwent a successful bone marrow transplant for leukemia, receiving stem cells from an unrelated and incompletely matched donor, and the same scenario developed after the transplant?
   Would the availability of a twin brother of the recipient or the donor be more helpful?

CASE 4
LYMPHOCYTOTOXICITY – Must read posted articles to understand the clinical implications of these 2 cases.

A. A patient underwent an operation for a large carcinoma of the colon. The resected tumor was analyzed by routine immuno-staining and array

   1. Based on the data and conclusions of the Science paper by Galon, et al who used array
technology to study a large group of patients with carcinoma of the colon, speculate on why the presence of intra-tumoral T cells might, **BUT NOT ALWAYS**, augurs a better prognosis.

Explain how array analysis can be used to define more precise prognoses for patients with cancer. If you weren’t a believer in how arrays will change your professional life, you will become one now!

B. A second patient noted a large, dark skin lesion on his anterior thigh. It was resected and regional draining lymph nodes were removed. This tumor and lymph nodes underwent array analysis. The lymphocytes in the melanotic tumor are attacking it...right?

C.

1. After reading the *Journal of Immunology* article and the *Perspective* assigned for today’s small group, you should be convinced that *conventional histology* can be very misleading.

   Discuss the specifics of that belief.

2. In patients with metastatic melanoma, increased intra-tumor CD4.25 FoxP3 cells seem to correlate with **shorter** survival than patients with metastatic disease that don’t have them in the tumor.

   Be ready to speculate on how a tumor could make a rogue T reg and how you might be able to switch it back to an effective anti-tumor T cell.

3. There are many immunologic anti-tumor strategies being considered by oncologists. Engineering a “new” T cell receptor is one way. Recently a spectacular example of this strategy was published in the New England J of Medicine (posted on HDwebsite).

   1. How did the presence of an acute leukemia present the opportunity to exploit cell surface molecules on lymphocytes?

   2. Describe how understanding T cell cytotoxicity and the principles of co-stimulatory lymphocyte responses came together as a “perfect immunologic storm” and culminated in a possible cure of a patient’s advanced leukemia.

   3. Discuss the immunologic and clinical implications of the down side of this specific therapy

4. Discuss at least 3 other approaches to an immunologic control or eradication of a malignancy.
KEY CONCEPTS AND LEARNING OBJECTIVES

You will be able to explicate the communication that exists between the nervous, neuro-endocrine, and immune systems.

To obtain competence for this lecture you will be able to:

   a. List the neuroendocrine molecules that have immune-modulating capabilities.
   b. Diagram the multi-directional, brain-immune connection.
   c. Understand the reciprocal linkages (neural and hormonal) that connect the brain to the immune system.
   d. Describe how cytokines contribute to sickness behavior.
   e. Understand the potential consequences of psychological and physical stress on the immune system and the implications for health and illness.
Content Summary

Introduction

Nervous System to the Immune System

Hormones, Neuropeptides, and Neurotransmitters with Effects on the Immune System

How Psychological Stress Affects the Immune System

Types of Stress

Immune System to the Nervous System

Sickness Behavior: Effect of the Immune System on the Nervous System

Implications of Stress on the Immune System

PNI and the Salubrious Nature of Stress
INTRODUCTION

- The focus of psychoneuroimmunology (PNI) is upon the mechanisms whereby the nervous system (including the brain, neurotransmitters and the neuroendocrine system) and the immune system (including lymphoid organs, cell types, and cytokines) communicate with one another.

- Results in this area have provided the biological basis by which to understand the health implications of neural-immune links, in particular, the effects of psychological constructs (including feelings, emotions, behavior, personality, and mind) on the immune system.

- These effects can be difficult to interpret and sometimes are controversial.

- Still, PNI research offers insight into mind-body mechanisms that over time may provide the basis for innovative approaches to disease prevention and symptom management.

NERVOUS SYSTEM TO THE IMMUNE SYSTEM

- The connection of the central nervous system (CNS) to the immune system involves two pathways:
  - Direct (neuronal)
  - Indirect (neuroendocrine).

  The most direct (neuronal) is the innervation of primary (thymus, bone marrow) and secondary (e.g. spleen) lymphoid organs as well as the adrenal medulla. In an indirect (neuroendocrine) manner, the CNS communicates hormonally with the immune system.

- These connections are activated by physical and/or psychological stressors that cause the release of neuropeptides and neurotransmitters in the brain such as:
  - Catecholamines, epinephrine (EPI) and norepinephrine (NE)
  - Gamma amino benzoic acid (GABA)
  - Acetylcholine (ACH)
  - Serotonin.

  These stimulate cells in the paraventricular nucleus (PVN) of the hypothalamus to synthesize and release corticotrophin releasing hormone (CRH) into the portal blood system of the pituitary. See Figure 1.

- In the anterior lobe of the pituitary gland, CRH stimulates the synthesis and release of adrenocorticotropic hormone (ACTH) into the peripheral circulation.
ACTH ultimately causes the release of the glucocorticoid (cortisol) from the adrenal gland (adrenal cortex) into the circulation.

- Most cells of the immune system are sensitive to cortisol and are inhibited in their function by this hormone.

Figure 1. Overview of the neuroendocrine interaction of the nervous system with the immune system.

- Peripheral nerves can also stimulate primary and secondary lymphoid tissue.
  - Bone marrow is primarily stimulated by noradrenergic fibers (secreting norepinephrine)
  - Thymus is stimulated by noradrenergic, cholinergic (secreting acetylcholine, ACH) and peptidergic fibers (secreting neuropeptides).
  - The spleen is strongly noradrenergic.
  - Lymph nodes receive noradrenergic and peptidergic stimulation.
  - Contact between lymphoid cells and nerve endings is synaptic-like.

Moreover, the adrenal medulla is innervated directly by sympathetic nerve fibers (with ganglia in the hypothalamus). When stimulated, the hypothalamus activates the splanchnic nerves, which in turn trigger chromaffin cells of the adrenal medulla to secrete catecholamines (epinephrine and norepinephrine) into the bloodstream. See Figure 2.
- T- and B- lymphocytes, neutrophils, mononuclear cells, and NK-cells possess receptors for catecholamines, ACH, and neuropeptides. Their effect on lymphoid tissue is dependent on the type of cell receiving the signal.

Figure 2. Overview of the direct effect of the nervous system on the immune system.

HORMONES, NEUROPEPTIDES, AND NEUROTRANSMITTERS WITH EFFECTS ON THE IMMUNE SYSTEM

- Cortisol
- Epinephrine and norepinephrine
- Beta-endorphins
- Enkephalins

- Cortisol is best known for its metabolic effects (increasing gluconeogenesis), anti-inflammatory effects (reducing cytokine production, T and B cell reactivity and NK cell activity) and its' ability to modulate the processing of information from the sense organs.

- Epinephrine and norepinephrine act as neurotransmitters in the CNS and are released into the circulation by the adrenal medulla, increasing leukocyte mobilization and resulting in increased NK cell activity. Epinephrine and norepinephrine are involved in emotions like fear and fright.

- Endorphins originate from a precursor molecule called pro-opiomelanocortin
(POMC), which is synthesized in the pituitary after CRH stimulation, but can also be synthesized by immune competent cells. Endorphins play an important role in analgesia and feelings of happiness (euphoria). (In the pituitary the POMC molecule is enzymatically split into the secretory products ACTH, and endorphin.)

- Enkephalins are produced in the brain, pituitary, and adrenal gland (simultaneously with epinephrine and norepinephrine) when stimulated, and play a role in analgesia. They can bind to the same opioid receptors as endorphins.
- Endorphins and enkephalins increase T cell reactivity and NK cell activity.
- Endorphins act more like hormones, while enkephalins act more like neurotransmitters.

HOW STRESS AFFECTS THE IMMUNE SYSTEM

- Stress is one of the most frequently mentioned psychological factors that may modulate the immune system.
- Stress is conceptualized as either a stimulus (i.e., stressor) or a response (i.e., stress response).
- An individual appraises a situation as either a challenge or a threat (stressor), dependent upon the individual’s adaptive capabilities (resources, social support, coping abilities, etc.).
- Any demand-capability imbalance leads to the perception of stress and the stress response (i.e., activation of the nervous system and the neuroendocrine pathways described above).
- Stressors can differ in type (physical or psychological), intensity, and duration (acute versus chronic).

TYPES OF STRESS

Acute controllable emotional or mental stress.

Chronic uncontrollable negative stress.

Acute controllable emotional or mental stress.

- Experiments with parachute jumping have shown immediate increases in the numbers of circulating leukocytes, in particular NK cells. The short lasting stress-related immune modulation in these situations is associated with increased
catecholamine levels.

- Catecholamines seem to place the immune system on an enhanced activation status and belong to the “fight or flight” reactive pattern.

When individuals were continuously monitored (10-minute intervals) before and after parachute jumping:

These individuals exhibit increased:

- Heart Rate
- Cortisol
- Epinephrine
- Norepinephrine. See Figure 4.

- Circulating mononuclear cells
  - T cells (CD3)
  - Helper T cells (CD4)
  - Cytotoxic T cells (CD8)
  - Monocytes (CD16)
  - NK cells (CD56).
- NK cell activity. See Figure 5.

The increase in NK cell activity is due to the increase in the number of circulating NK cells. The effect on circulating NK cells is reproduced by the administration of epinephrine or norepinephrine.

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**Figure 4.** Heart rate, serum cortisol and catecholamine levels before, and immediately after, and 1 hour after parachute jumping.
Figure 5. Overview of the effects of the nervous system on the immune system.

One hour after parachute jumping, the number of various mononuclear cell populations decreases because the cells have localized to the regional lymph nodes. The effect is mimicked by the administration of catecholamines and cortisol. See Figure 6.
Figure 6. Catecholamine (Epi) and cortisol (Cort) induced localization of mononuclear cells to the lymph nodes.

Immediate leukocyte mobilization in the circulation is due to catecholamines (epinephrine and norepinephrine). Under the influence of catecholamines and cortisol, leukocytes redistribute to the lymph nodes where they can respond quickly to antigenic (infectious) challenge. [Redistribution to the lymph nodes is a consequence of hormonal modification of adhesion molecules on the surface of the leukocytes (increased expression of CD11a) and activation of cognate adhesion molecules on the surface of endothelial cells (ICAM-1). After the stressful event, hormone levels return to normal and leukocyte numbers return to normal in the circulation. See Figure 7.

Figure 7. Redistribution of blood mononuclear cells after stress.
The impact of leukocyte redistribution on the immune response can be profound. See **Figure 8.** The acute effects have a positive impact on immune function as measured by delayed type hypersensitivity (DTH) reaction to intradermal injection of antigen. Low cortisol concentrations (as a consequence of acute stress) of 5 mg/kg markedly enhance DTH responses. Interestingly, higher concentrations of 40 mg/kg depress DTH responses. Moreover, chronic stress (extending for several days or weeks) actually decreases immune responsiveness as judged by DTH.

**Figure 8.** Effect of cortisol on delayed type hypersensitivity (DTH).

**Chronic uncontrollable negative stress.**

- Chronic uncontrollable negative stress like **caregiving** to the chronically ill (e.g. Alzheimer's patients) can cause immune suppression.

Individuals who have given care for several years to Alzheimer's patients were monitored over an extended period for their levels of stress and for their capacity to mount an immune response in comparison to a matched control group of individuals who were not chronically stressed.

The caregivers as a group exhibited:

- Decreased cytokine production (IL-1).
- Decreased antibody production, judged by enzyme linked immunoadsorbent
When divided into high stress and low stress groups, the high stress group exhibited:

- Decreased cytokine response to influenza vaccine (Fluzone) as judged by cytokine production (IL-2).
- An increase in the numbers of colds.

**Figure 9.** Immunological and symptom effects of chronic stress.

**IMMUNE SYSTEM TO THE NERVOUS SYSTEM**

Cytokines produced as a consequence of an on-going immune or inflammatory process can have direct effects upon the CNS. These cytokines are:

- IL-1
- TNF
- IL-6.

- Cytokines change the firing frequencies of nerve cells in the CNS and influence the secretion of neuroendocrine factors of the hypothalamus-pituitary-adrenal gland axis, especially ACTH production.
- Receptors for cytokines have been found in the CNS and pituitary gland.
Furthermore, leukocytes are capable of neuropeptide and neurotransmitter production. Activation of T- and B-lymphocytes can stimulate these cells to produce:

- ACTH
- beta-endorphin
- Enkephalins.

The production of endorphins and enkephalins by activated immune cells may induce an analgesic effect in infected tissue.

The production of these cytokines, hormones, neurotransmitters, and neuropeptides may modulate on-going immune or inflammatory responses and may also influence/induce behavioral changes, known as sickness behavior.

**SICKNESS BEHAVIOR: EFFECT OF THE IMMUNE SYSTEM ON THE NERVOUS SYSTEM**

Activation of the immune system by an infectious agent induces the production of cytokines (IL-1, IL-6, and TNF), which in addition to activating the immune system also send signals to the CNS that modify behavior. This behavior modification is known as "sickness behavior". See **Figure 10**.

![Figure 10](image-url). Overview of the effects of the immune system on the nervous system.
- Symptoms like fever, headache, muscle and joint pain, diminished appetite, lethargy, are a consequence of these cytokines and are characteristic of sickness behavior.

- Cytokines produce these symptoms by two known means:
  - Via the circulation
  - Via afferent neurons (i.e., vagal).

- Via the circulation, cytokines cross the brain blood barrier most likely through the circumventricular organs (CVO) and neurons in this area express receptors for IL-1, TNF, and IL-6. Interaction of these cytokines with their cognate receptors results in neural system activation and the production of prostaglandins.

- Via the vagal afferents, IL-1 produced by leukocytes stimulates the related regions of the brain.

- Thus these molecules, as part of the immune system, alert the brain to infection or injury, communicating the body's distress.

**IMPLICATIONS OF STRESS ON THE IMMUNE SYSTEM**

Effective immune responses require significant mobilization of energy and resources.

The immediate stress response is also associated with the mobilization of energy to enable the body to deal with a threat or danger. In a 'fight or flight' situation, energy is diverted to the muscles, heart rate increases, and the body is made ready for physical action and increased sensory performance.

The mobilizations of energy for immune function and for fight or fight responses, however, are roughly opposite. Energy cannot be diverted in both directions simultaneously. So after the immediate release of catecholamines, which mobilize the leukocytes of the immune system, the immune response is temporarily suppressed by the slower release of cortisol, in order to maximize energy for the fight or flight response.

This is achieved by cortisol production (that is normally used by the body to slow down immune system activity).

This is a highly adaptive response to short term stressors where the body could soon resume normal immune function. It becomes disadvantageous when stress responses are maintained over longer periods of time.
PNI AND THE SALUBRIOUS NATURE OF STRESS

Although the word ‘stress’ generally has negative connotations, stress is a familiar aspect of life, being a stimulant for some, but a burden for others. It is often overlooked that an acute stress response can have salubrious (health promoting) effects. The duration of a stressor is thought to be important when considering its impact on individuals. The timing of the stressor and the nature of the stressor (acute versus chronic) determine the magnitude of the immune response. **Differences exist in the ways that acute (short-term) and chronic (long-term) stressors affect the immune system.** For example, chronic stress significantly suppresses delayed-type hypersensitivity (DTH) responses (e.g. to skin test) and decreases leukocyte mobilization to the skin. Acute stress enhances the DTH response and increases leukocyte mobilization.

So although **stress is typically thought to be immunosuppressive**, acute stress can result in immune enhancement that can promote protection from infections, but alternatively could contribute to the exacerbation of immunopathology. In response to immunological challenge, acutely stressed individuals show significantly greater leukocyte infiltration, enhanced production of chemokines (chemoattractant proteins for leukocytes), IL-1, IL-6, TNF and IFN gamma (proinflammatory and Th1 cytokines). Further, acute stress enhances maturation and trafficking of dendritic cells (DCs) to sites of antigenic challenge. DCs are efficient antigen presenting cells (APCs) and these APCs effectively promote the activation and recruitment of T lymphocytes during initial antigen challenge, inducing a long-term increase in immunologic memory. The result would be subsequent augmentation of the immune response during secondary antigen exposure. Thus, the evolutionarily adaptive fight-or-flight stress response may prepare the immune system for impending danger (e.g. infection and wounding by a predator). The result would be enhanced immunological protection from the infectious agent and more rapid healing of the wound. However, **stress could also exacerbate a pathological condition that is worsened by an increased immune responsiveness such as an autoimmune response or an inflammatory insult or disease condition.** Moreover, inopportune stress could contribute to the initiation of a pathological or autoimmune condition if it occurred at an inopportune period of significant insult.

An acute stress-induced enhancement of immune function provides a selective advantage when viewed from an evolutionary perspective. The brain perceives a stressor, warns of danger and promotes survival. Stress-responsive neurotransmitters and hormones are the brain's signals to the body. Since stressful natural encounters could result in wounding and infection, it is likely that stress experienced at the time of immunological challenge promotes an antigen-specific immune response. It is possible that the differential effects (**immune enhancement or immune suppression**) may be due to differences in glucocorticoid sensitivity or receptivity of the immune response that may depend on the phase (early versus late) of the response. At the beginning of an immune response, factors such as leukocyte trafficking, antigen presentation, helper T cell function, leukocyte proliferation, cytokine and chemokine function and effector cell function may be receptive to stress hormone-mediated immune enhancement. In contrast, at a later stage, these components may
be more receptive to immune suppression. When an animal is wounded during an escape from a predator, the animal is acutely stressed during the chase, after which it is exposed to antigens and pathogens that enter its wounds. The stressor experienced during such a condition is acute and proximal to the time of immunological challenge. The physiological changes accompanying acute stress have adaptive immune enhancing effects that are established early during an immune response and may be clinically beneficial to understand and harness. **The effects of stress on immune function is especially relevant because stress is a ubiquitous fact of life and stress hormones are important components of an individual's physiological response to the environment.**

**STUDY QUESTIONS**

1. What is the role of the immune system in the way that stress relates to illness?
2. What is the evidence that stress can increase the risk of infectious illness?
3. What mechanisms mediate the association between stress and illness?

**EXAMPLE OF TEST QUESTION**

Cytokines responsible for the fever, headache, muscle and joint pain, diminished appetite, lethargy, and weakness associated with infection are:

A. IL-1, IL-6, TNF.
B. IFN alpha, IFN beta, IFN gamma.
C. G-CSF, M-CSF, GM-CSF.
D. IL-12, IL-15, IL-18.
E. IL-4, IL-5, IL-10.

**CORRECT ANSWER TO ABOVE QUESTION: A**
Further reading for anyone who is interested:

**Psychological Stress and Disease**
Sheldon Cohen; Denise Janicki-Deverts; Gregory E. Miller
http://jama.ama-assn.org/cgi/content/full/298/14/1685
Psychoneuroimmunology
H. L. Mathews

Introduction

Psychoneuroimmunology (PNI) is the study of the means by which the nervous system and the immune system communicate with one another.

It is a theoretical construct by which to understand how physiological and psychological events can impact immune function.

Nervous System to the Immune System

• The connection of the central nervous system (CNS) to the immune system involves two pathways:
  - Direct (neuronal)
  - Indirect (neuroendocrine)
Physical and/or psychological stressors cause the release of neuropeptides and neurotransmitters in the brain:

- Catecholamines, epinephrine (EPI) and norepinephrine (NE)
- Gamma amino benzoic acid (GABA)
- Acetylcholine (ACH)
- Serotonin

Fight or Flight Response
Peripheral nerves can also stimulate primary and secondary lymphoid tissue.

- Bone marrow is primarily stimulated by noradrenergic fibers (secreting norepinephrine)
- Thymus is stimulated by noradrenergic, cholinergic (secreting ACH) and peptidergic fibers (secreting neuropeptides)
- The spleen is strongly noradrenergic
- Lymph nodes received noradrenergic and peptidergic stimulation.

**Hormones, Neuropeptides, and Neurotransmitters with Effects on the Immune System**

- Cortisol
- Epinephrine and norepinephrine
- Beta-endorphins
- Enkephalins
Effects on the Immune System

- Cortisol:
  - anti-inflammatory
  - reduces cytokine production
  - T and B cell reactivity
  - NK cell activity

- Epinephrine and norepinephrine:
  - increase leukocyte mobilization

- Beta endorphins and Enkephalins:
  - increase -T cell reactivity
  - NK cell activity

How Does Stress Affect the Immune System

Stress is often considered to modulate immune function.
Stress is either a stimulus (i.e., stressor) or a response (i.e., stress response).
A situation is either a challenge or a threat (stressor).
If the stressor causes a demand-capability imbalance, the results is the perception of stress and a stress response.
Stressors are: (physical or psychological) (acute or chronic).

Types of Stress

- Acute controllable emotional or mental stress (parachute jumping)
- Chronic uncontrollable negative stress (care giving to the chronically ill, e.g. Alzheimer’s patients)
Individuals parachute jumping exhibit increased:

- Heart Rate
- Epinephrine
- Norepinephrine
- Cortisol
- Circulating Mononuclear cells
  - T cells (CD3)
  - Helper T cells (CD4)
  - Cytotoxic T cells (CD8)
  - Monocytes (CD16)
  - NK cells (CD56)
- NK cell activity
Parachute Jumping

Heart Rate

Epinephrine

Minutes

mg/mL
Individuals parachute jumping exhibit increased:

- Heart Rate
- Epinephrine
- Norepinephrine
- (Cortisol)
- Circulating Mononuclear cells
  - T cells (CD3)
  - Helper T cells (CD4)
  - Cytotoxic T cells (CD8)
  - Monocytes (CD16)
  - NK cells (CD56)
- NK cell activity
Parachute Jumping

Epinephrine and Cortisol Induced Localization

Cellular Redistribution
Impact of Leukocyte Redistribution on an Immune Response

Delayed Type Hypersensitivity (DTH)

Tuberculin
Effect of Cortisol

![Graphs showing effect of cortisol on DTH response](images)

- **CORT (5 mg/kg)** vs **control**
  - Days after induction: 0, 1, 2, 3, 4, 5, 6
  - DTH % Increase: 0 to 60

- **control** vs **CORT (40 mg/kg)**
  - Days after induction: 0, 1, 2, 3, 4, 5, 6
  - DTH % Increase: 0 to 40
Types of Stress

- Acute controllable emotional or mental stress (parachute jumping)

- Chronic uncontrollable negative stress (care giving to the chronically ill, e.g. Alzheimer’s patients)
Caregivers for Alzheimer’s patients exhibit:

- Decreased cytokine production (IL-1).
- Decreased antibody production.

When divided into high stress and low stress groups, the high stress group exhibited:

- Decreased cytokine response to influenza vaccine (IL-2).
- Increased number of colds and “flu”.
Effects of Chronic Stress

Cytokines produced as a consequence of an on-going immune or inflammatory process can have direct effects upon the CNS. These are:

- IL-1
- TNF
- IL-6

Leukocytes can produce neuropeptides and neurotransmitters including:

- ACTH
- Beta-endorphin
- Enkephalins
**Sickness Behavior**

Effect of the Immune System on the Nervous System

Activation leads to the production of cytokines-

- IL1, IL-6, TNF.

Resulting in-

- fever, headache, muscle and joint pain
- diminished appetite, lethargy.

Cytokines produce sickness behavior by two mechanisms:

- Via the circulation.
- Via afferent neurons (i.e. vagal).

Alerting the brain to infection or injury.
Implications of Stress on the Immune System

- An effective immune response requires energy.
- Immediate stressor requires energy for fight or flight situation.
- Therefore an immediate release of catecholamines-mobilizing leukocytes.
- Cortisol-temporary suppression to divert energy to the fight or flight response.
- Disadvantageous only when stress is chronic.
LEARNING GOALS
You will understand how the immune system maintains tolerance to self and what the clinical implications are when tolerance is lost.
To achieve these goals, you will be able to:

- Understand how genes can predispose a patient to autoimmune disease
- Identify antibodies can cause autoimmune disease
- Identify a new subset of T cells that are associated with autoimmune disease
- Explain how normal or "well-intended" host reactions can predispose one to an autoimmune disease.
- Understand how T regulator cells are critical to prevention of autoimmune disease
- Develop a conceptual approach to identifying sites in the immune response that may be clinically manipulated to limit the clinical expression of autoimmune diseases

BACKGROUND READING
1. Janeway 7th edition: 610-614;620-622. This reading was previously assigned for the "perturbations" lecture. Do NOT memorize any table in the text. If I want you to know some concept of a specific disease it will be mentioned either in the lecture, lecture notes or small groups.
2. The posted articles on the HD website

DEVELOPED BY
John A. Robinson, MD
HOW TO SUCCEED IN SMALL GROUPS

Before coming to class:

1. Read assigned chapters/pages and develop answers for ALL the questions in the 4 clinical vignettes

During the Small Group Session:

2. Each small group (should be 4-5 peers - please do not sort yourselves into large groups - you will learn much less) should discuss the four case studies and decide the best solutions to the specific integrating questions associated with each case.

3. After approximately an hour of discussion by the subgroups, the facilitator will recapitulate the answers to the integrating questions by selecting a subgroup to present a synthesis of their relevant discussions to the entire group. Facilitators will select, at their discretion, a small group for the discussion of the individual cases.

4. History has shown that students who don’t contribute to the Small Groups do not do well in the Course (remember that about 25-30% of the final comes from small groups!) and also have been assaulted by their fellow group members

5. At the end of the session, a master answer sheet will be posted on the Host Defense website.
1. **AUTOIMMUNE ENDOCRINE DISEASES**

**SCENARIO 1A (a true story)**
A group of residents and attending physicians were relaxing late one night at a local bistro. One attending, an internist almost certainly, noted that their waitress had rather prominent eyes and a tremor. One thing led to another and he found her pulse to be 150 and regular and very large mass in her anterior neck. He referred her to an endocrinologist at Loyola. Two days later, she was evaluated. The patient reported increasing irritability, tremors and weight loss. The only other significant history was that her sister had a “bad thyroid” and her mother had rheumatoid arthritis. Physical examination revealed a blood pressure of 160/55 and a pulse of 140. She had a fine tremor of her fingers, velvety smooth skin and a firm, very large thyroid. Auscultation over the gland revealed a loud bruit. Blood analysis revealed a thyroid hormone level of 24ug/dl (normal 4-12) and her thyroid stimulating hormone (TSH) was almost undetectable.

**QUESTIONS**

1. Does the patient’s gender and family history tell us anything about the etiology of the disease? If so, what genes would be likely suspects and how could they mediate the development of an autoimmune disease?

2. A specific antibody is usually the mediator of this disease; what is its antigenic specificity and isotype and how does it produce the disease? T cells are also mandatory participants in the disease process. What, if any, antigenic specificities might they have and how do they drive the process?

3. How might a tissue microarray of her thymus tissue differ from that of a normal female thymus? Speculate how a tissue array of her thyroid differs from a normal thyroid.

**SCENARIO 1B**
An eight year old male from Coxsackie, NY developed fever, diarrhea and then profound diabetic ketoacidosis. There was almost no detectable insulin in his peripheral blood at a time when his blood glucose was 900 mg% (normal less than 126%). Autoantibodies to a virus, glutamic acid decarboxylase (GAD) and insulin were detected in his blood.

**QUESTIONS**

1. Walk through the possible immunologic reactions by which this patient developed diabetes. What is (are) the targets, what attacked it(them) and what systems failed, if any?
2. If this patient had X-linked agammaglobulinemia (remember that disease from a prior small group?), would it still be possible that he could develop diabetes? What does this astute observation in the New England Journal article tell you about the significance of detecting autoantibodies in a patient's blood.

2. AUTOIMMUNE NEUROMUSCULAR DISEASE

A 24 year male noted that he was unable to keep up with his teammates at their Saturday pickup basketball game. On the subsequent Monday, he was unable to rise from bed because of weakness. 24 hours later he was admitted from the Emergency Room to a Medical Intensive Care unit. Shortly thereafter he had to be placed on a mechanical ventilator because he was unable to move his muscles of respiration adequately. After a muscle biopsy (which did not reveal any infiltrating inflammatory cells), he then underwent plasmapheresis (removal of plasma proteins) and within 24 to 48 hours could be weaned from ventilatory support. He was discharged asymptomatic 7 days later. An immunologist in the Medical School was given the bag of plasma removed during the plasma pheresis. 5ml of this plasma was infused into mice and these animals, within 4-6 hours, were unable to crawl about their cage.

QUESTIONS

1. The plasma apparently contained a factor(s) that caused weakness. What proteins are the most likely suspects and is a plasma factor specific for something that will cause muscle weakness?

2. Are you surprised that the muscle biopsy showed no cells? What immunohistopathologic studies might be helpful in understanding this disease?

3. Patients with an auto-immune inflammatory muscle disease called polymyositis have symptoms of muscle weakness somewhat like the basketball player but their biopsies show muscle necrosis and lymphocytes. How is the pathogenesis of polymyositis different than the disease the basketball player had?

4. Understanding this disease is an example of how clinical application of basic research findings and technology can be combined to provide for improved patient care-Can you explain how an eel is involved?
3. Gastrointestinal autoimmune diseases. Have to read the posted article to understand this case.

**Case History**: An eighteen year old female developed bloating and severe diarrhea during "rush week" at Parttee University. She had no significant past medical history other than a vaguely remembered time in middle school when she also had diarrhea and severe weight loss. The latter was so severe that her parents thought she had an eating disorder and had her evaluated by a psychiatrist. She became asymptomatic after her mother "changed her diet for a while". Her current physical examination in the university infirmary was not remarkable. She had no lymphadenopathy and no rash. Her stool was tested for infectious parasites and found to be negative for all pathogens. The initial bloodwork revealed multiple autoantibodies. A procedure was performed in the GI lab and the biopsy is shown below. A biopsy from a normal is shown on the left.

![Biopsies](image)

1. What procedure was done and what is the diagnosis? Is the history typical for your diagnosis and what autoantibodies were most likely detected?

2. Are the autoantibodies in this disease pathogenic or diagnostic or both? Would looking for cytokine expression possibly be helpful? And which ones might you look for? You can actually get ahead of the clinical immunology literature if you think this one through!

3. Now understanding the immunology of her diagnosis, can you explain the history, past and present, and; can you develop a strategy for treatment that will allow her to drink beer at rush week next year (maybe)?
4. Other routes to autoimmune disease- You have to read the posted articles to understand the concepts fully. If the basic defect is known, the clinician can predict much of the patient’s disease expression.

There are 3 well described genetic defects associated with severe autoimmune clinical phenotypes.

a. Those with **FoxP3 mutations** have immunodysregulation, polyendocrinopathy, enteropathy and the transmission is X-linked (IPEX syndrome). Speculate on why they have hormonal deficiencies and what cell may be missing from their repertoire.

b. Those with **AIRE defects** have polyendocrinopathy, candidiasis (a fungal infection), and ectodermal dysplasia (APECED syndrome). Speculate on why these patients have low calcium in their blood, are infertile, have deep voices and abnormal skin and bone formation.

c. Patients with a **defect in Fas mediated apoptosis** have markedly increased size of their lymph nodes and spleen and produce huge amounts of IgG antibodies. Speculate why they have more lymphoid tissue than normal and why they have an unusual number of CD3+, CD4- & CD8- cells circulating in their peripheral blood.
THE BIG PICTURE

IMMUNE........

DEFECTS, DEFICIENCIES AND DYSFUNCTION - HOW THEY CAN LEAD TO DISEASE STATES

Do not remember any specific TLRs for this test
The Integrated Immune Response

The type of Infection dictates the type of Immune response

The CD4 family

TMMI LOGIC

- Parasites over time became increasingly successful at surviving inside macrophages. Increased survival led to cell death and tissue destruction.
- TMMI is a survival response to pathogens that can survive inside cells.
- The first upgrade that the immune system developed to cope with this threat was to provide a means by which the infected macrophage could signal that not only was it infected but also what it was infected with—this was the advent of the antigen presenting cell.
- In parallel with this ability, it created the T lymphocyte, a cell that could specifically recognize the call for help and once activated could do something about it.
- These T cells developed the capability of activating macrophages and making them more effective killers.
TMMI LOGIC

• Antibody responses will not be successful because the parasite is inside the cell and antibody won’t have access to it
• The availability of a helper T cell that could recognize a specific infection and then recruit and activate many killer cells that, in turn, could phagocytize and kill the parasite was the only option
• The parallel ability to suppress any misguided attempts to mount a antibody response was developed to conserve energy

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**Th1 Initiation steps**

![Diagram of Th1 initiation steps]

**WHAT SHOULD HAPPEN DURING A Th1 RESPONSE**

![Diagram showing Th1 activation]

- INF-γ
- HYDROLASES & OXIDASES
- MHC

- INF-γ, IL-12, IL-6, IL-8
- TNF-α, IL-1

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An unregulated Th-1 response or scenario when macrophages cannot kill effectively

An unregulated Th-1 response or scenario when superantigens are the driver

Fig 8.43 © 2001 Garland Science
The host response will be the complete opposite if the patient cannot generate a TMMI

- No activated macrophages
- Lots of happy intracellular bugs or non-processed complex antigens
- The defect may be genetic or drug induced
- Be able to predict the possible site of defect based on what you know about TMMI

CYTOTOXIC RESPONSES

- If a parasite can be phagocytized and its antigens re-presented to T cells then TMMI can be an effective response.
- What about infections of non-APC type cells and mutations of host cells?- here the TMMI strategy might not be effective
- The first line of defense against mutated or infected cells was the development of the natural killer lymphocyte that could recognize the normal MHC on the cell surface was no longer normal

NK cells

- NK cells sample cell surfaces and as long as they sense a normal MHC-I they keep their inhibitory receptors "on"
- Absence or abnormal conformations of the MHC-I however turn the inhibitory receptors "off" and turn on their cytotoxic modes
- NK kill by direct cytotoxicity and also by using...NEW CONCEPT...FcR that can sense that a specific antibody has bound to a cell-this signals the immune system recognizes something on that cell as an antigen and the cell needs to be eliminated. (this mechanism was likely an "add-on later in the scheme of things"
- NK have important suppressive roles in the pregnant uterus
The CD4 & Cytotoxicity

- CD8 + Th1
- Th2
- CD4

T Cell Cytotoxicity

- The advent of viruses, with their capability of infecting almost every somatic cell in the host, probably forced the issue of needing something better than the NK cell.
- That upgrade was the development of the CD8 T cell—a cell that reflects all the characteristics of the adaptive system.
- The CD8 also exploited the availability of the T helper cell to amplify its killing efficiency.
- CD8 also does not require co-stimulation signal after initial activation—why?

Cytotoxicity

- These cells do not Have B7 on surface!
What happens to CD8 cytotoxicity when Th1 response is defective?

- NK
- CD4
- DC
- CD8

ALTERED MHC-I

VIRAL-INFECTED SOMATIC CELL

IL-2, IL-12

INF-γ

IL-21

CD8 ACT

KILL

VIRAL-INFECTED SOMATIC CELL

SEVERE CLINICAL ILLNESS

The B Cell Response is a dominant Th2 response

- Antibodies are required in infections or disease where the pathogen and/or toxins are not readily phagocytosed by APC and are not produced endogenously and displayed on cell surfaces. This type of infection is called extracellular.
- The B cell was developed to counter this threat. This cell captures antigen in the extracellular environment by displaying its specific antigen receptor on its cell surface.
- Exploiting the existing concept of T cell helper functions, the B cell internalizes the captured antigen, redispays it in MHC-II and activates Th2 helper cells that were built to strongly promote antibody production and suppress any misguided attempts at Th1 reactions which will not be helpful against an extracellular threat.

The CD4 family

- Th0
- IL-12
- T-Bet
- Th1
- IL-23
- TGF-β
- IL-4
- GATA-3
- Th2
- Treg
- FoxP3
- Th17
- ROR
- T-Bet
NORMAL RESPONSE TO A PATHOGEN ANTIGEN
WHEN A B CELL IS THE ANTIGEN PRESENTING CELL

THE LOGIC OF THE Th2/B Cell RESPONSE

FcR review
Inappropriate or deficient B cell responses

Type III hypersensitivity diseases are immunoexcess, not immunodeficiency states

ABNORMAL RESPONSE TO A B-CELL ANTIGEN WHEN THE B CELL IS ABSENT OR DEFECTIVE

EXTRACELLULAR ANTIGEN E.G., BACTERIAL TOXIN

NO EFFECTIVE AG PRESENTATION
NO B CELLS AVAILABLE FOR GROWTH
NO PLASMA CELLS
NO ANTIBODY PRODUCTION
NO FACILITATED PHAGOCYTOSIS
RESULTS INFECTIONS WITH BACTERIA PROTECTED BY CAPSULES
ABNORMAL RESPONSE TO A B-CELL ANTIGEN
WHEN THE T CELL IS ABSENT OR DEFECTIVE

EXTRACELLULAR ANTIGEN
E.G., BACTERIAL TOXIN

- EFFECTIVE AG PRESENTATION WILL OCCUR
- ISOTYPE SWITCHING & GROWTH
- NO IL-4, IL-5, IL-10
- NO B CELLS
- NO PLASMA CELLS
- NO IgG ANTIBODY PRODUCTION (HYPER IgM SYNDROME)
- NO FACILITATED PHAGOCYTOSIS
- RESULT: INFECTIONS WITH BACTERIA PROTECTED BY CAPSULES

TYPE I HYPERSENSITIVITY: ALLERGY
Allergen diverts Ig Response to IgE

Th0

- NO IL-12
- OR allergy-TLR
- OR mast cell/basophil

NO IL-12 or allergy-TLR or mast cell/basophil

RICH SOURCE OF IL-4

ALLERGY
The second response to allergen

Th0

- NO IL-12
- OR allergy-TLR

HIGH SOURCE OF IL-4

IL-4, 10, 13, IL-5, eotaxin

Enzymes
Mast cell
Viral Infection—The Ultimate Test

- Viruses pose the ultimate challenge to the host because they infect a wide range of somatic cells and APC, and can disseminate infection either cell to cell or via the blood.
- The immune system invokes all its strategies to combat viral infections

Viral Infections
The basic strategy

- Use NK cells as a first line of defense
- Employ a short burst of TMMI in response to viral infection or uptake by DC.
  - This will jump start the helper system but is risky because protracted TMMI has the potential for widespread collateral damage
- Activate the CD8 system that will target infected cells only
- Activate the B Cell system to produce anti-viral antibody that will suppress blood borne viral dissemination
- Promote development of T and B cell memory systems that will prevent significant re-infection with the same virus

Why a B cell response is necessary in a viral infection

- B cells produce antibodies that neutralize viruses in the cytosol.
- Th2 cells produce cytokines that support B cell activation.
- PC cells differentiate into memory B cells and plasma cells that secrete antibodies.
- UNINFECTED CELLS

NK cells can also play a role in the early detection and elimination of viral-infected cells.
EXAMPLE of viral ingenuity: Sites that can be exploited to allow viral survival (much more efficient than being a superantigen!)

They can even meddle with TLRs

- Viruses can upload bacterial lipopolysaccharides that inhibit the TLR on DC that will recognize them and activate the immune system
Th17 immunity, the good and the bad

- Generated by certain organisms, especially fungi
- Activated by unique trio of cytokines: IL-6, TGF-β, and IL-23
- Strongly suppressed by IL-4 and/or IFN-γ
- Identified by unique nuclear activating factor: ROR

The Bad: Chronic inflammation & autoimmunity

Th17 immunity

- Certain Fungi & bacteria
- "IL-17" TLR
- IL-23
- IL-6, TGF-beta
- Fibroblast

The CD4 family
The CD4 family

The T regulator lymphocyte

- Most T regs express the unique transcription factor FoxP3 and are CD3,4,25+
- They arise in the thymus and in the periphery, are governed by critical levels of cytokines, esp. TGF-β and AIRE
- They are dependent upon exogenous IL-2 for survival & proliferation
- They express cytotoxic T lymphocyte antigen, hereafter known as CTLA-4 on their surface
- CTLA-4 can suppress T cell activation by competing with CD28 and down regulating CD80/86 on DC
- CTLA-4 expression is controlled by FoxP3 and CTLA-4 gene is highly polymorphic
- T regs can also dampen reactions with IL-10

The T regulator lymphocyte

- T regs can arise in the thymus, in regional lymph nodes and other peripheral sites (especially the gut).
- Th0 cells are converted at peripheral sites during ongoing immune reactions when local cytokine concentrations begin trending to increased TGF-β, and decreased IL-6
- As TGF-β becomes dominant, FoxP3 upregulates CTLA-4 which then shuts down APC
Core Concepts of Autoimmunity

- Tregs are trained either in the thymus or are adaptive—that is, they develop during an immune response to control it.
- A loss of tolerance will almost always involve a CD4,25,FoxP3 (Treg) abnormality.
- Autoimmune disease may be secondary to a deficiency or malfunction of Tregs that allows proliferation of Th17 promoted inflammation.
- Some Immunodeficiencies are associated with hyper-Treg function.
- It is likely that whoever controls Tregs may control the immunology world and be able to modulate many diseases.
Central and Peripheral Tolerance Mechanisms in the Adaptive Immune System.

Major Reasons for Highly Variable Immune Responses (ergo: clinical) to a infection

- Polymorphic MHC
- Polymorphism of cytokine genes
- Polymorphism of TLR genes
- Polymorphism of CTLA4
- And probably many other factors
Normal T&B cell response to vaccine antigens and induction of active immunity

Reaction to vaccine based on hyper B/T cell response secondary to manner of MHC presentation and TLR/cytokine poly-morphisms

No immune response to vaccine antigens because of MHC lack of effective presentation and/or TLR/cytokine mutation/suppression

Autoimmune response to self antigen

Th17 autoimmunity

The multiple faces of Autoimmunity

- Expression is a mix of gender, age, genes, environment—a quantitative trait expressed as loss of tolerance
- CD4,25,Fox3P regulators fail & Th17 cells and Th17 dominate
  - Failure: IL-2 low; TGF/IL6 ratio low
- Autoantibodies can stimulate hormone production
- Autoantibodies can block/downregulate action
- Cytolytic attack can destroy critical cells
Real World Manipulation of the Immune Response—Tumors

- The basic tenets of tumor immunology are:
- Some but not all malignant cells look "different" to immune cells. The ones that look different can be attacked by immune effector cells.

Where the immune system can fail in a patient with a malignancy

- Tumor cell
  - Recruits and
  - Converts LY
  - To protect it!
IMMUNITY to INFECTION

Date:  5/11/12
Reading Assignment: None.

KEY CONCEPTS AND LEARNING OBJECTIVES

You will be able to identify the role of the immune system and its components in protection against infectious agents.

To attain competence for this lecture you will be able to:

a. List the effector mechanisms for the control of bacterial infections.
b. List the effector mechanisms for the control of viral infections.
c. Contrast host defense for extracellular and intracellular microorganisms.
d. Identify the three main classes of interferons.
Content Summary

Introduction

Immune Resistance to Infectious Disease

The Generalized Response to Bacteria

The Generalized Response to Viruses

Immunity to Toxin Producing Microorganisms

Different Immune Effector Mechanisms in Host Defense Protect Against Different Microbial Pathogens

Extracellular Microorganisms

Intracellular Microorganisms

Interferons
INTRODUCTION

- Most infectious agents do not penetrate the body surface. They are prevented by a variety of biochemical and physical barriers. See Figure 1.

- Upon penetration of the body the interaction of the immune system with infectious organisms is a dynamic interplay of host mechanisms aimed at eliminating infections and microbial strategies designed to permit survival in the face of powerful host effector mechanisms.

- Different types of infectious agents stimulate distinct patterns of immune response, which are protective and result in specific immunity.

- The principal protective immune response against bacteria in extracellular space and in plasma consists of specific antibodies that opsonize bacteria for phagocytosis by macrophages and neutrophils. Specific antibody and complement can also lyse gram negative bacteria and opsonize gram positive bacteria. See Figures 2 and 3.

- Specific antibodies neutralize toxins produced by bacteria. See Figure 3.
Intracellular bacteria are capable of surviving and replicating within host cells, including phagocytes, because they have developed mechanisms for resisting lysosomal degradation.

Immunity against intracellular bacteria is principally cell-mediated and consists of CD4+ Th1 cells that activate macrophages by the production of cytokines. The characteristic pathologic response to infection by intracellular bacteria is granuloma formation. See Figure 4.
- Viruses are obligatory intracellular microbes.
- Innate immunity against viruses is mediated by cytokines and NK cells. See Figure 5.
- Adaptive immunity against viruses consists of specific cytotoxic T lymphocytes (CTLs). CTLs effectively lyse infected cells and may contribute to tissue injury even when the infectious virus is not cytopathic by itself. Adaptive immunity against viruses also consists of specific antibodies, which are synthesized prior to T cell mediated killing of infected cells. See Figure 6.

![Figure 5](image1.png)

Figure 5. Protection from viruses. Figure 2.55 in the text.

![Figure 6](image2.png)

Figure 6. Protection by cytotoxic T lymphocytes. Figure 1.27 in the text.
IMMUNE RESISTANCE TO INFECTIOUS DISEASE.

The sequence of infectious challenge followed by host response is depicted in Figure 7.

- Immediate host defense is available quickly (in less than an hour) after microbial invasion. Macrophages are resident in almost all tissues and are found in particularly large numbers at mucosal sites. Neutrophils are present in the blood in very large numbers and they can be rapidly recruited to any site. Macrophages and neutrophils possess receptors, which enable them to bind and phagocytose microorganisms.

- During the early phase of host defense, antigen presenting cells carry peptides (resulting from degradation of proteins from the infecting microorganism) to the local lymph node and present them to T cells. This process allows the rare (1 in a million) antigen specific T cells to encounter the presented peptide-MHC complex as the T cells traffic through the lymph node.

- Antigen-specific B cells acquire antigen via their surface Ig receptor, process and present this antigen via their MHC class II molecules. The presented antigen-MHC complex is available for activated T cells to recognize.

- The activation of T cells per se leads to the clonal proliferation of antigen-specific cells and to the production of effector T cells, such as Th1, Th2 cells, and CTLs. The interaction of specific Th cells with B cells leads to the generation first of a primary and then secondary antibody response.

Figure 7. Infectious challenge and immune response. Figure 10.2 in the text.
Specific antibody clearly plays an important role in clearing many primary infections. The presence of IgM begins to be detectable at about 3-4 days after antigen entry and peaks between 2-3 weeks. The IgG response is delayed by about 5-7 days and persists much longer. CTL production follows the production of Ab. Immunological memory results. See Figure 8.

![Figure 8](image)

**Figure 8. Host protection and immunological memory. Figure 10.1 in the text.**

THE GENERALIZED RESPONSE TO BACTERIA is summarized in Table 1.

<table>
<thead>
<tr>
<th>Immune System Component</th>
<th>Target</th>
<th>Effector Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils and macrophages</td>
<td>Intact microorganism</td>
<td>Phagocytosis and intracellular destruction</td>
</tr>
<tr>
<td>Antibody</td>
<td>Surface antigen of a microorganism</td>
<td>Blocks adsorption of the microorganism to host tissue</td>
</tr>
<tr>
<td>Antibody + Complement</td>
<td>Surface antigen of a microorganism</td>
<td>Enhanced phagocytosis by neutrophils and macrophages</td>
</tr>
<tr>
<td>Antibody + Complement</td>
<td>Host cell surface antigen</td>
<td>Lysis of infected cell</td>
</tr>
<tr>
<td>Activated Macrophages</td>
<td>Engulfed microorganism</td>
<td>Enhanced intracellular destruction</td>
</tr>
</tbody>
</table>
Additional immune protective mechanisms exist for protection from viruses. See Table 2.

THE GENERALIZED RESPONSE TO VIRUSES is summarized in Table 1 above with additional anti-viral mechanisms in Table 2.

Table 2. Direct Mechanisms by which the Immune Response Combats Viral but not Bacterial Infectious Agents

<table>
<thead>
<tr>
<th>Immune System Component</th>
<th>Target</th>
<th>Effector Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytotoxic T lymphocytes</td>
<td>Cell surface presented viral peptides</td>
<td>Cytolysis</td>
</tr>
<tr>
<td>Natural killer cells</td>
<td>Virally infected host cells</td>
<td>Cytolysis</td>
</tr>
<tr>
<td>Interferons</td>
<td>Non-virally infected host cells</td>
<td>Cellular anti-viral state</td>
</tr>
</tbody>
</table>

IMMUNITY TO TOXIN PRODUCING MICROORGANISMS.

- In diseases caused by exotoxigenic organisms (e.g., Clostridium tetani toxin causes tetanus), the function of the immune response is not only to eliminate the invading organism but also to neutralize any toxin.

- This neutralization occurs as the result of the antibody blocking the combination between the toxin and its target. (The toxin blocks inhibitory neuron action leading to chronic muscle contraction.) See Table 3.

Table 3. Direct Mechanism of Anti-Bacterial but not Anti-Viral Immunity

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Toxins</th>
<th>Neutralization by antibody binding</th>
</tr>
</thead>
</table>

DIFFERENT IMMUNE EFFECTOR MECHANISMS IN HOST DEFENSE PROTECT AGAINST DIFFERENT MICROBIAL PATHOGENS

The immune system must cope with a spectrum of pathogenic microorganisms, which have distinct lifestyles and different arms of the immune system are needed for protection from different microorganisms. In addition, many pathogenic microorganisms have evolved specific counter measures, which limit or inhibit the effectiveness of the immune response. We can categorize the types of pathogens as follows:
- Extracellular microorganisms
  - bacteria
  - eukaryotes
    - single celled (fungi)
    - multicellular (parasites)

- Intracellular organisms
  - bacteria
  - protozoa
  - viruses

EXTRACELLULAR MICROORGANISMS

- **Bacteria.** These are probably the simplest types of organism to combat and in many cases phagocytes are able to clear the infection. Specific antibody is highly effective, both by directing complement lysis (gram-negative bacteria) and inducing opsonization and phagocytosis (gram negative and gram-positive bacteria). [A good example of a gram-negative bacteria is *Neisseria meningitidis*, which causes meningitis. A good example of a gram-positive bacteria is *Staphylococcus aureus*, which causes skin infections.] Some bacteria [for example *Streptococcus pneumoniae* that causes pneumonia] have evolved anti-phagocytic capsules, which prevent recognition by innate mechanisms and require both antibody and complement to promote efficient clearance by phagocytes. IgA plays an important role for organisms that infect mucosal surfaces (respiratory tract, gut, genito-urinary tract). [Secretory IgA can protect from *Neisseria gonorrhoea*, which causes gonorrheae.]

- **Fungi.** Phagocytic cells primarily handle these microorganisms, particularly cytokine activated phagocytes. [An excellent example of a fungus is *Candida albicans*, which is a dimorphic fungus that causes yeast infections.]

- **Parasites.** Large, multicellular parasites present a special problem to the immune system and indeed are rather poorly eliminated. The mechanisms deployed include antibody directed complement attack and antibody dependent cellular cytotoxicity (ADCC - antibodies specific for the parasite are bound by their Fc region to receptors on the effector cell) mediated by eosinophils. See Figure 9. Innate immunity is generally ineffective. The parasites employ many evasion strategies including complement inhibitors, release of large quantities of soluble antigen (decoy) and acquisition of host proteins.

![Figure 9. Eosinophils attacking Schistosoma mansoni by way of IgE mediated ADCC. Figure 9.33 in the text.](image)
INTRACELLULAR MICROORGANISMS

- **Bacteria and Protozoa.** Many microorganisms have evolved resistance to the constitutive killing mechanisms used by phagocytes. These pathogens actively replicate inside bacteria, either in the phagosome or in the cytoplasm. [An excellent example is *Mycobacterium tuberculosis*, which causes tuberculosis.] These types of microorganisms cannot be eliminated by the innate immune system. T cell activation is required and a Th1 response is necessary for clearance of the microorganism. Antibody is generally ineffective in eliminating the infection.

- **Viruses.** Viruses are a very diverse group of obligate intracellular pathogens. Almost every form of immunity comes into play against some type of virus. Enveloped viruses can be damaged by complement attack. Phagocytes can take up and destroy antibody and complement coated viruses. Other key players in antiviral immunity are; interferons (IFN) (See Table 4.), NK cells, antibody, and CTL taken in temporal order during an infectious encounter. [Influenza virus is an excellent example, this virus cause the “flu”.

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Target</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-alpha</td>
<td>Leukocytes</td>
<td>Virus infected cells</td>
<td>Block viral replication</td>
</tr>
<tr>
<td>IFN-beta</td>
<td>Fibroblasts</td>
<td>Virus infected cells</td>
<td>Block viral replication</td>
</tr>
<tr>
<td>IFN-gamma</td>
<td>T lymphocytes</td>
<td>Macrophages</td>
<td>“Activation” MHC class I &amp; class II increase</td>
</tr>
</tbody>
</table>

**INTERFERONS**

The IFN are a family of related glycoproteins produced by a variety of cell types in response to viral infection (IFN-alpha is produced by leukocytes and IFN-beta is produced by essentially any cell in the body, e.g. fibroblasts) or antigenic stimulus of Th1 cells (IFN-gamma). When a leukocyte or fibroblast cell is infected by a virus, the infected cell releases Type I IFN (either alpha or beta depending upon the cell population). Type I IFN will diffuse to the surrounding cells. When it binds to receptors on the cell surface of adjacent cells, those cells begin the production of proteins that prevent the synthesis of viral proteins. This prevents the spread of the virus throughout the body. See Figure 10. (IFN-gamma is Type II IFN and is produced by Th1 lymphocytes.)
The antiviral effect of interferons is as follows. Interferon stimulates the synthesis of two enzymes: a synthetase and a protein kinase.

The synthetase catalyzes the polymerization of adenine nucleotides into a long chain of adenine units. This oligonucleotide activates ribonuclease to degrade viral mRNA.

The protein kinase transfers a phosphate group to initiation factor EIF-2 that is involved in protein synthesis. The phosphorylated form of EIF-2 cannot assist in the formation of the initiation complex for protein synthesis and the viral mRNA remains untranslated. The viral mRNA is attacked by ribonuclease and degraded.

IFN-gamma is produced as a cytokine by antigen activated T lymphocytes and serves to activate macrophages to enhanced levels of cytotoxicity.

Figure 10. Antiviral effect of Type I Interferons.
There is no reading assignment but for those who are interested in materials related to this presentation see:

Figures: (Unless otherwise noted) **Janeway’s Immunobiology**, 7th Edition, Murphy et al., Garland Publishing.

### STUDY QUESTIONS

For each of the following infections, toxins, pathogens, or disorders, indicate the primary immune response(s) and effector mechanism(s) involved (defensive and pathological). Be specific about the types of cells and/or humoral factors implicated. (See page 440 in Janeway’s Immunobiology, 7th Edition.

- Influenza virus infection.
- *Candida albicans* infection.
- *Staphylococcus aureus* infection.
- *Neisseria meningitidis* infection.
- *Clostridium tetani* toxin intoxication.
- *Mycobacterium tuberculosis* infection.

### EXAMPLE OF TEST QUESTION

1. Mechanisms of anti-viral immunity include all of the following EXCEPT:
   
   a. Antibody and complement mediated viral lysis
   b. Antibody and complement mediated opsonization for enhanced phagocytosis
   c. Antibody neutralization of toxins
   d. Intracellular destruction by activated macrophages
   e. Natural killer cells

CORRECT ANSWER TO ABOVE QUESTION: C
IMMUNITY TO INFECTION

INTRODUCTION

• Most infectious agents do not penetrate the body surface. They are prevented by a variety of biochemical and physical barriers.

“INFECTION”

“Infectious Agents = Examples of Specific Antigens” Focus on the immune response as presented in the lecture handout.
Different types of infectious agents (that are pathogens) stimulate distinct patterns of immune response.

Principle protective immune responses against extracellular bacteria consists of specific antibodies that opsonize with complement the microorganism for macrophage and neutrophil phagocytosis.

Toxins produced by such bacteria are also neutralized and eliminated by specific antibodies.
Staphylococcus aureus
(extracellular bacteria)

Neisseria meningitidis
(extracellular bacteria)

Erythrogenic toxin
(Streptococcus pyogenes)

Streptococcus pneumoniae
(extracellular bacteria)
**Streptococcus pyogenes** - Disease

- Scarlet Fever
- Produced Toxin

*Staphylococcus aureus* (extracellular bacteria)
Disease – Impetigo/Boils

- Superficial infection of skin
- Usually
  - *Staphylococcus aureus*
- Initially → vesicles
- Later crusts

*Neisseria meningitidis* (extracellular bacteria)
Gram Stain of Spinal Fluid  
Disease - Meningitis

Streptococcus pneumoniae
extracellular bacterium

Disease - Pneumonia

Streptococcus pneumoniae
Typical Pneumonia
Immunity against intracellular microbes is cell-mediated and consists of CD4+ T cells that activate macrophages (as in delayed type hypersensitivity).

- **Mycobacterium tuberculosis**

![Image of infected and activated macrophages with Mycobacterium tuberculosis](asm.microbelibrary.org/copyright)

**Infected macrophage**
- Lysosome
- Mycobacterium
- Antigen

**Activated infected macrophage**
- Lysosome
- Mycobacterium
- Antigen
Mycobacterium tuberculosis
Disease - tuberculosis

Acid fast sputum
Acid fast tissue
Aurimine fluorescence
Colony morphology
Lowenstein-Jensen medium
• Viruses are obligatory intracellular microbes.
• Innate immunity against viruses is mediated by interferon and NK cells.
• Adaptive immunity against viruses consists of specific antibodies during the course of infection.
• The major defense mechanism against established viral infection is virus specific CTLs and antibodies.

INFLUENZA
Disease- Flu
Influenza virus
IMMUNE RESISTANCE TO INFECTIOUS DISEASE

Local infection, penetration of epithelium
Macrophages
Local infection of tissues
Complement activation
Dendritic cells migrate to lymph nodes
Phagocytes act on infected cells
Adaptive immunity initiated by engaging dendritic cells
Protection against infection
Infection caused by specific antibodies
T-cell dependent macrophage activation and cytokine T cells
- Immediate host defense is available instantly or within an hour or so after microbial invasion. Macrophages and neutrophils can be rapidly recruited to any site.

- Antigen-presenting cells carry peptides to the local lymph node and present them to T cells. This process allows the rare T cell that recognizes the antigen to encounter the presented peptide-MHC complex as the T cell traffics through the lymph node.

- Antigen-specific B cells acquire antigen via their surface Ig receptor, process, and present this antigen to T cells.

- Activation of T cells leads to the clonal proliferation of antigen-specific cells and to the production of effector T cells, such as TH1, TH2 cells, and CTLs.

- IgG begins to be detectable at about 2-4 days after antigen entry and peaks between 3-5 weeks. The IgG response is delayed by about 6-7 days and persists much longer.

- Immunological memory results.
Table 2. Direct Mechanisms by which the Immune Response Combats Bacterial and Viral Infectious Agents

<table>
<thead>
<tr>
<th>Immune System Component</th>
<th>Target</th>
<th>Effector Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils and macrophages</td>
<td>Inact microorganism</td>
<td>Phagocytosis and intracellular destruction</td>
</tr>
<tr>
<td>Antibody</td>
<td>Surface antigen of a microorganism</td>
<td>Blocks adsorption of the microorganism to host tissue</td>
</tr>
<tr>
<td>Antibody + Complement</td>
<td>Surface antigen of a microorganism</td>
<td>Enhanced phagocytosis by neutrophils and macrophages</td>
</tr>
<tr>
<td>Antibody + Complement</td>
<td>Host cell surface antigen</td>
<td>lysis of infected cell</td>
</tr>
<tr>
<td>Activated Macrophages</td>
<td>Encapsulated microorganism</td>
<td>Enhanced intracellular destruction</td>
</tr>
</tbody>
</table>

In diseases caused by exotoxigenic organisms (e.g., Clostridium tetani causes tetanus), the function of the immune response is not only to eliminate the invading organism but also to neutralize any toxin.

This neutralization occurs as the result of the antibody blocking the combination between the toxin and its target. (The toxin blocks inhibitory neuron action leading to chronic muscle contraction.)
**Clostridium tetani**

- Tetanus (Lock Jaw)
- Neurotoxin acts on nerves, resulting in the inhibition of muscle relaxation
- Tetanospasmin - “spasms” or “Lock Jaw”

Tetanospasmin inhibits the release of acetylcholine by interfering with activity of the cholinesterase that normally breaks down acetylcholine.

Table 3. Direct Mechanism of Anti-Bacterial but not Anti-Viral Immunity

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Toxin</th>
<th>Neutralization by antibody binding</th>
</tr>
</thead>
</table>

**DIFFERENT IMMUNE EFFECTOR MECHANISMS IN HOST DEFENSE PROTECT AGAINST MICROBIAL PATHOGENS**
• Extracellular microorganisms
  • Bacteria
  • Eukaryotes
  • Single celled (fungi)
  • Multicellular (parasites)

• Intracellular organisms
  • Bacteria
  • Protozoa
  • Viruses

EXTRACELLULAR MICROORGANISMS

• Bacteria.
  • Neisseria meningitidis
  • Staphylococcus aureus
  • Streptococcus pneumoniae

• Fungi.
  • Candida albicans

• Parasites.
  • Schistosoma mansoni
**EXTRACELLULAR MICROORGANISMS**

- **Bacteria.**
  - Phagocytes
  - Specific antibody
  - Plus complement = opsonization
  - Mucosal - IgA

- **Fungi.**
  - Cytokine activated phagocytes

- **Parasites.**
  - Specific antibody
  - Complement
  - Antibody dependent cellular cytotoxicity
Schistosoma mansoni – infection of the gut

INTRACELLULAR MICROORGANISMS

- Bacteria and Protozoa.
  - Mycobacteria tuberculosis
- Viruses.
  - Influenza
**INTRACELLULAR MICROORGANISMS**

- Bacteria and Protozoa.
  - Activated T cells
  - Th1 cytokines
  - Results in activated macrophages

- Viruses.
  - Essentially all forms of immunity
  - Antibody, complement, phagocytes, Th1 cytokines, CTL, NK cells, interferons

**INTERFERONS**

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Target</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α</td>
<td>Leukocytes</td>
<td>Virus infected cells</td>
<td>Block viral replication</td>
</tr>
<tr>
<td>IFN-β</td>
<td>Fibroblasts</td>
<td>Virus infected cells</td>
<td>Block viral replication</td>
</tr>
<tr>
<td>IFN-gamma</td>
<td>T lymphocytes</td>
<td>Macrophages, APCs</td>
<td>“Activation” MHC class I &amp; class II increase</td>
</tr>
</tbody>
</table>
STUDY QUESTIONS

• For each of the following infections, toxins, pathogens, or disorders, indicate the primary immune response(s) and effector mechanism(s) involved (defensive and pathological). Be specific about the types of cells and/or humoral factors implicated. (See page 440 in Janeway’s Immunobiology, 7th Edition.)

• Influenza virus infection
• Candida albicans infection
• Staphylococcus aureus infection
• Neisseria meningitidis infection
• Clostridium tetani toxin intoxication
• Mycobacterium tuberculosis infection
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• Staphylococcus aureus
• Neisseria meningitidis
• Clostridium tetani
• Mycobacterium tuberculosis

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IMMUNITY TO “INFECTION”