BCHEM 471
Immunology

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AIM

- To appreciate how components of the immune system work to protect against development of clinical disease.

- The basic systems and cells involved in immune responses will be presented to give a general overview of functional immunity.

- Components and systems will be defined to allow an understanding of concepts of innate and adaptive responses, and how these responses interact with one another to form the basis for protection against infections.

Importance of Immunology

- A) Vaccines
- B) Recombinant DNA technology
- C) Monoclonal and Polyclonal Antibody technology
- D) Improving transplant outcomes
- E) Treatment outcomes
- F) Global eradication of diseases

Industrial Application

- Biomedical Research Institutions
- WHO, CDC
- Research Universities
- Pharmaceutical companies
- Ministry of Health/ Hospitals
- Vertinary centers
- Biomed and Biotech companies
- Forensic institutions

Salary Range

- ✓ 48,000-60,000 GHC p/a
- ✓ 80,000-120,000 USD p/a

What is Immunology?

- Immunology is defined at the study of the immune system.

- The immune system is a system of cells, tissues and their soluble products that recognizes, attacks and destroys agents that could be harmful to the health of an individual or organism.

- In principle the normal functioning of immune system lead to ***immunity***.

What is Immunity?

- The Latin term ***immunitas***, meaning "exempt," gave rise to the English word ***immunity***, which refers to all the mechanisms used by the body as protection against environmental agents that are foreign to the body.

- These agents may be microorganisms or their products, foods, chemicals, drugs, pollen, or animal hair and dander.

- The concept of immunity dates back to 1500s, before the cause of disease was understood. Survivors after exposure to deadly disease were referred to as ***except from*** or ***immune.***

Pioneers of Immunology

- **Edward Jenner** (1796), one of the pioneers who solidified the birth of immunology

- Jenner observed that dairymaids and farmers lacked the pock-marked complexions of their fellow citizens, and wondered whether those who worked with cattle might be resistant to smallpox because of their close contact with livestock.

- Cows of that era often suffered from cowpox disease, a disorder similar to smallpox but much less severe.

- In an experiment that would be prohibited on ethical grounds today, Jenner deliberately exposed an 8-year-old boy to fluid from a cowpox lesion.

- Two months later, he inoculated the same boy with infectious material from a smallpox patient.

- In this first example of successful vaccination, the boy did not develop smallpox. Jenner's approach to smallpox prevention was quickly adopted in countries throughout Europe.

ROBERT KOCH

- In 1884, Robert Koch proposed the "germ theory of disease,"

- Which stated "that microbes invisible to the naked eye were responsible for specific illnesses"

- The first human disease-causing organisms or **pathogens** were identified in the late 1800s

- At about the same time, Louis Pasteur applied Jenner's immunization technique for smallpox to the prevention of various animal diseases.

- Pasteur demonstrated that inoculation with a pathogen that had been weakened in the laboratory could protect against a subsequent exposure to the naturally occurring pathogen.

- It was Pasteur who coined the term **vaccination** (from the Latin *vaccinus*, meaning "derived from cows") for this procedure, in honor of Jenner's work.

- The research of Pasteur and other investigators spurred the evolution of immunology as a science distinct from (but related to) the established fields of microbiology, pathology, biochemistry and histology

Today, **immunology** can be defined :

As the study of the cells and tissues that mediate immunity and the investigation of the genes and proteins underlying their function.

Smallpox Immunization

Smallpox infection in Bangladesh girl in 1973

Edward Jenner vaccinating James Philips against smallpox

Number of countries with one or more cases per month
30
15
0
smallpox officially eradicated
1965
1970
1975
1980
Year

What is the nature of Immune Response

- **A normal healthy person's body always strives to maintain homeostasis, a natural state of balance of all its organs**

- **When this homeostasis is disturbed by pathogens or the deregulation of body cells (as in cancer), the immune system responds in an attempt to restore balance.**

- At its simplest level, this response involves identifying and clearing damaged and dying cells from the body.

- More complex responses have evolved to counteract assault on the body by infectious agents, including bacteria, viruses, parasites and fungi.

- Because these foreign invaders are literally everywhere on Earth and constantly seeking vulnerable hosts, the immune system is constantly occupied with containing attacks.

- Humans are surrounded by other organisms-in the air, in the soil, in the water, on the skin, and on the **mucosae**, the protective layers of epithelial cells that line the gastrointestinal, urogenital and respiratory tracts.

- While most of these organisms are harmless and some are even beneficial, some are pathogenic.

- Like all species, pathogens live to reproduce. In order to reproduce, however, many must penetrate a host's body or one of its component cells.

What is infection?

- **Infection** is defined as the attachment and entry of a pathogen into the host.

- Once inside the body or cell, the pathogen replicates, generating progeny that spread into the body in a localized or systemic fashion.

- The manner of this replication determines whether the pathogen is considered extracellular or intracellular.

Extracellular pathogens

- Certain bacteria and parasites do not need to enter cells to reproduce.

- After accessing the body, these organisms replicate first in the interstitial fluid bathing the tissues and may then disseminate via the blood.

Intracellular pathogens

- Pathogens such as viruses and other bacteria and parasites, enter a host cell, subvert its metabolic machinery, and cause it to churn out new virus particles, bacteria, or parasites.

- These pathogens may then also travel systemically by entering the blood.

	Extracellular		Intracellular	
	Interstitial spaces, blood, lymph	Epithelial surfaces	Cytoplasmic	Vesicular
Site of infection				
Organisms	Viruses Bacteria Protozoa Fungi Worms	*Neisseria gonorrhoeae* *Streptococcus pneumoniae* *Vibrio cholerae* *Helicobacter pylori* *Candida albicans* Worms	Viruses *Chlamydia* spp. *Rickettsia* spp. Protozoa	*Mycobacterium* spp. *Yersinia pestis* *Legionella pneumophila* *Cryptococcus neoformans* *Leishmania* spp.
Protective immunity	Complement Phagocytosis Antibodies	Antimicrobial peptides Antibodies, especially IgA	NK cells Cytotoxic T cells	T-cell and NK-cell dependent macrophage activation

The immune system protects against four classes of pathogens		
Type of pathogen	**Examples**	**Diseases**
Extracellular bacteria, parasites, fungi	*Streptococcus pneumoniae* *Clostridium tetani* *Trypanosoma brucei* *Pneumocystis carinii*	Pneumonia Tetanus Sleeping sickness *Pneumocystis* pneumonia
Intracellular bacteria, parasites	*Mycobacterium leprae* *Leishmania donovani* *Plasmodium falciparum*	Leprosy Leishmaniasis Malaria
Viruses (intracellular)	Variola Influenza Varicella	Smallpox Flu Chickenpox
Parasitic worms (extracellular)	*Ascaris* *Schistosoma*	Ascariasis Schistosomiasis

Types of Immune Response

- The mammalian immune system can mount two types of responses:
- **Innate response** is triggered by disruptions to homeostasis caused by either non-infectious or infectious means
- **Adaptive response** that is composed of highly specialized, systemic cells and processes that eliminate or prevent pathogen growth

Mechanisms Underlay Immune Responses

- There were two schools of thought on what mechanisms underlay immune responses.

- One group of scientists believed that immunity depended primarily on the actions of cells that destroyed or removed unwanted material from the body. This clearance process was referred to as **cell-mediated immunity**.

- However, another group of researchers was convinced that soluble molecules in the serum of the blood could directly eliminate foreign entities without the need for cellular involvement. In this case, the clearance process was referred to as **humoral immunity**, a term derived from the historical description of body fluids as "humors."

- Today, we know that both **cell-mediated** and **humoral responses** occur simultaneously during an immune response and that both are often required for complete clearance of a threat.

- The cells responsible for cell-mediated immunity are collectively called **leukocytes** or white blood cells.

- The soluble molecules responsible for humoral immunity are proteins called **antibodies,** and antibodies are secreted by a particular type of leukocyte. In this case a B cell (plasma cell).

- Antibodies protect against extracellular pathogens and their toxic products

Specific antibody
Bacterial toxins
Bacteria in extracellular space
Bacteria in plasma
cell with receptors for toxin
macrophage
Neutralization
Opsonization
Complement activation
+
complement
Ingestion by macrophage
Ingestion by macrophage
Lysis and ingestion

Humoral and Cell-mediated Immunity

Cells of the Immune System

TABLE 2-1 Normal Blood Cell Counts

	Mean Number per Microliter	Normal Range
White blood cells (leukocytes)	7400	4500–11,000
Neutrophils	4400	1800–7700
Eosinophils	200	0–450
Basophils	40	0–200
Lymphocytes	2500	1000–4800
Monocytes	300	0–800

Cell		Activated function
Macrophage		Phagocytosis and activation of bactericidal mechanisms Antigen presentation
Dendritic cell		Antigen uptake in peripheral sites Antigen presentation
Neutrophil		Phagocytosis and activation of bactericidal mechanisms

Cell		Activated function
Eosinophil		Killing of antibody-coated parasites
Basophil		Promotion of allergic responses and augmentation of anti-parasitic immunity
Mast cell		Release of granules containing histamine and active agents

Cell		Activated function
Macrophage		
		Phagocytosis and activation of bactericidal mechanisms Antigen presentation

Cell		Activated function
Dendritic cell		
		Antigen uptake in peripheral sites Antigen presentation

DCs were first characterized by Ralph Steinman. Dr. Steinman was awarded a share in the 2011 Nobel Prize in Physiology or Medicine for his seminal work.

Cell		Activated function
Neutrophil		
		Phagocytosis and activation of bactericidal mechanisms

Neutrophils or PMN: polymorphonuclear leukocytes

Cells in blood stream, not in healthy tissues

Become attracted by activated macrophages

Neutrophil Recruitment

Crawling Neutrophil chasing bacterium

David Rogers, Vanderbilt University

Neutrophil extracellular traps

Behaviour of neutrophils that formed NETs *in vivo*

Mod. from Kolaczkowska et al. (2013), *Nat Rev Immunol*, 13, 159-175

Cell		Activated function
Eosinophil		
		Killing of antibody-coated parasites

Contain granula with argini-rich basic proteins, which can be stained by eosin

In peripheral tissue, low numbers in blood

After activation, they release granula with toxic substances that kill microorganisms esp. parasites but can also damage host in allergy; release cytokines to attract other cells

Cell		Activated function
Basophil		
		Promotion of allergic responses and augmentation of anti-parasitic immunity

Function similar to Eosinophils (common precursor) but differentiation is mediated by other cytokines (e.g. IL-3)
low numbers in blood, recruitment and activation by cytokines, Fc receptor
recognizes Ab-conjugated antigens, secretion of toxic granules

Introduction to Innate Immune response

Features of Innate Immune Response

- Barrier Defense
- Complement Activation
- Pattern Recognition
- Inflammation
- Phagocytosis

Innate Immunity

Barriers of the Host Immune System

	Skin	Gut	Lungs	Eyes/nose/ oral cavity
Mechanical	Epithelial cells joined by tight junctions			
	Longitudinal flow of air or fluid		Movement of mucus by cilia	Tears Nasal cilia
Chemical	Fatty acids	Low pH Enzymes (pepsin)	Pulmonary surfactant	Enzymes in tears and saliva (lysozyme)
	β-defensins Lamellar bodies Cathelicidin	α-defensins (cryptdins) RegIII (lecticidins) Cathelicidin	α-defensins Cathelicidin	Histatins β-defensins
Microbiological	Normal microbiota			

Distribution of lymphoid tissues in the body

The distribution of lymphoid tissues in the body

Primary lymphoid tissues

Secondary lymphoid tissues

MALT, BALT
GALT
NALT
SALT

Primary and Secondary Lymph organs

Thymus

Spleen

Lymph node

Bone Barrow

Organization of a lymph node

Protection Against Infection

Protection against infection			
Normal flora Local chemical factors Phagocytes (especially in lung)	Wound healing induced Antimicrobial proteins and peptides, phagocytes, and complement destroy invading microorganisms	Complement, cytokines, chemokines, Phagocytes, NK cells Activation of macrophages Dendritic cells migrate to lymph nodes to initiate adaptive immunity Blood clotting helps limit spread of infection	Infection cleared by specific antibody, T-cell dependent macrophage activation and cytotoxic T cells

Infection triggers an inflammatory response

Pathogen Recognition

Antigen Presenting Cells

Dendritic cells link the Innate and the Adaptive immune system

Dendritic cells form the bridge between innate and adaptive immune responses

Infection→ Activation
Sensing infections (innate function)

Antigen → specific T cell
Activation (adaptive function)

Features of Adaptive Response

- Specificity
- Division of Labor
- Immunological Memory
- Tolerance (central and peripheral)
- Faster response

Innate and Adaptive Immunity

Phases of Immune Response

The course of a typical antibody response

Phases of the immune response			
Response		**Typical time after infection to start of response**	**Duration of response**
Innate immune response	Inflammation, complement activation, phagocytosis and destruction of pathogen	Minutes	Days
Adaptive immune response	Interaction between antigen-presenting dendritic cells and antigen-specific T cells: recognition of antigen, adhesion, co-stimulation, T-cell proliferation and differentiation	Hours	Days
	Activation of antigen-specific B cells	Hours	Days
	Formation of effector and memory T cells	Days	Weeks
	Interaction of T cells with B cells, formation of germinal centers. Formation of effector B cells (plasma cells) and memory B cells. Production of antibody	Days	Weeks
	Emigration of effector lymphocytes from peripheral lymphoid organs	A few days	Weeks
	Effector cells and antibodies eliminate the pathogen	A few days	Weeks
Immunological memory	Maintenance of memory B cells and T cells and high serum or mucosal antibody levels. Protection against reinfection	Days to weeks	Can be lifelong

Summary

Innate immunity (immediate: 0–4 hours)
Infection
Recognition by preformed, non-specific and broadly specific effectors
Removal of infectious agent
Early induced innate response (early: 4–96 hours)
Infection
Recruitment of effector cells
Recognition of PAMPS. Activation of effector cells and inflammation
Removal of infectious agent
Adaptive immune response (late: >96 hours)
Infection
Transport of antigen to lymphoid organs
Recognition by naive B and T cells
Clonal expansion and differentiation to effector cells
Removal of infectious agent

Questions

- What is the difference between infection and disease
- What is the major difference between extracellular and intracellular pathogens?
- Distinguish between cell-mediated and humoral immunity
- Describe 3 important features of adaptive immunity
- What are the two stages of tolerance ?
- Distinguish between PAMPs and PRR
- Is inflammation good or bad, and why?

References

1. Paul, W.E. (ed): Fundamental Immunology, 6th ed. New York, Lippincott Williams & Wilkins, 2012

2. Janeway Immunology (2016)

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4. Kuby Immunology, 2015

5. Cellular and Molecular Immunology, Abbas et al., 2015

Antigen Processing and Presentation

Dr. Alex Kwarteng

Pattern Recognition

- Pattern Recognition Receptors (PRR)
  - Toll-like receptors (TLRs)
  - NOD-like receptors (NLRs)
- Pathogen Associated Molecular Patterns (PAMPs)
- Death Associated Molecular Patterns
- (DAMPs)

Recognizing Danger

Origins of Innate Immunity ?

Late 1880's: Discovery of phagocytosis and phagocytes
cellular immunity

"mobile white corpuscles of the blood which absorb microbes and destroy them"

Ilya Mechnikov

The Nobel Prize in Physiology or Medicine 1908
Ilya Mechnikov, Paul Ehrlich

"in recognition of their work on immunity"

*"It has been shown that the white corpuscles (phagocytes) entertain no fear of **microbial poisons** (endotoxins, dead microbes and nucleic acids) and are well fitted to absorb them and make them harmless."*

How do phagocytes recognizes these danger signals?

"Toll" a gene important in embryogenesis in establishing the dorsal-ventral axis

Christiane Nüsslein-Volhard

"Das ist ja toll!"

The Nobel Prize in Physiology or Medicine 1995
Edward B. Lewis, Christiane Nüsslein-Volhard, Eric F. Wieschaus

"the genetic control of early embryonic development"

Toll-like receptor (TLR) family

- 13 TLRs identified in mammals (11 expressed in humans)
- Cell surface and endosomal TLRs
- Recognizing a diverse range of ligands

TLR dimerization

Antigen & Immunogen

- An antigen is any agent capable of binding specifically to components of the immune system, such as the B cell receptor (BCR) on B lymphocytes and soluble antibodies.

- An immunogen is any agent capable of inducing an immune response and is therefore ***immunogenic.***

- There are many compounds that are incapable of inducing an immune response, yet they are capable of binding with components of the immune system that have been induced specifically against them.

- Thus all immunogens are antigens, but not all antigens are immunogens.

Antigens

Most are proteins or large polysaccharides from a foreign organism.

- **Microbes:** Capsules, cell walls, toxins, viral capsids, flagella, etc.

- **Non-microbes**: Pollen, egg white , red blood cell surface molecules, serum proteins, and surface molecules from transplanted tissue.

- **Lipids and nucleic acids are only antigenic when combined with proteins or polysaccharides.**

Haptens

- A hapten is a compound that, by itself, is incapable of inducing an immune response (non-immunogenic) but against which an immune response can be induced when conjugated to a carrier.

Requirements for immunogenicity

- ❖Foreignness
- ❖High molecular size
- ❖Complexity of structure
- ❖Degradability
- ❖Antigenic determinants
- ❖Others, conformation, charge, dosage, route

Epitope

Small part of an antigen that interacts with an antibody.

- Any given antigen may have several epitopes.
- May be Linear or Conformational
- Each epitope is recognized by a different antibody.

Antigens/Epitopes

surface molecules of sufficient size, structural complexity

Microbial antigens/epitopes

Antibodies

- Made in response to exposure to the antigen, and react specifically to that antigen

- Proteins that recognize and bind to a particular antigen with very high *specificity*.

- One virus or microbe may have several *antigenic determinant sites*, to which different antibodies may bind.

- Each antibody has at least two identical sites that bind antigen: *Antigen binding sites*.

- Valence of an antibody: Number of antigen binding sites. Most are bivalent.

- Belong to a group of serum proteins called immunoglobulins (Igs).

- Antigen-antibody reactions
  - Antiserum: serum that contains a large amount of antibodies that bind to a particular antigen

- Natural antibodies
  - Igs which react specifically to antigens of no known exposure.
  - Usually of low titre but may confer resistance to certain infections.

Epitopes: Antigen Regions that Interact with Antibodies

©1999 Addison Wesley Longman, Inc.

Antibodies work in three ways:

1. **Neutralisation**. blocking the biological activity of their target molecule e.g a toxin, by binding to it's receptor
2. **Opsonisation**. interact with special receptors on various cells, including macrophages, neutrophils, basophils and mast cells allowing them to "recognise" and respond to the antigen
3. **Complement Activation**. cause direct lysis by complement complement recruitment also enhances phagocytosis

4. **Neutralization:** IgG inactivates viruses by binding to their surface and neutralize toxins by blocking their active sites.

5. **Antibody-dependent-cell-mediated cytotoxicity:** Used to destroy large organisms (e.g.: worms). Target organism is coated with antibodies and bombarded with chemicals from nonspecific immune cells.

6. **Complement Activation:** Both IgG and IgM trigger the complement system which results in cell lysis and inflammation.

All the interactions between antigen and antibody are non-covalent interactions:

- ionic interactions, eg. like attraction of opposite charges on amino acids;
- hydrogen bonds;
- hydrophobic interactions.

Protective Mechanisms of Antigen-Antibody Binding

© BENJAMIN/CUMMINGS

Types of antigens

- Exogenous antigens (Mostly expressed by extracelluar pathogens ie. parasites)
- Endogenous antigens (Expressed by intracellular pathogens, viruses & some bacteria)

- T-dependent antigens
- T- independent antigens

Figure 4.25 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Antigen Processing and Presentation

- Why is it needed? What is the purpose?
- How does it happen? & What are the components

- How are the pathways of endogenous and exogenous antigen kept apart?
- What are the consequences?
- (What happens after presentation & processing)

Antigen Presenting Cells

- Dendritic cells
- Monocytes/Macrophages
- B-cells

Cell		Activated function
Dendritic cell		
		Antigen uptake in peripheral sites Antigen presentation

DCs were first characterized by Ralph Steinman. Dr. Steinman was awarded a share in the 2011 Nobel Prize in Physiology or Medicine for his seminal work.

	Cytosolic pathogens	Intravesicular pathogens	Extracellular pathogens and toxins
Degraded in	Cytosol	Endocytic vesicles (low pH)	Endocytic vesicles (low pH)
Peptides bind to	MHC class I	MHC class II	MHC class II
Presented to	Effector CD8 T cells	Effector CD4 T cells	Effector CD4 T cells
Effect on presenting cell	Cell death	Activation to kill intravesicular bacteria and parasites	Activation of B cells to secrete Ig to eliminate extracellular bacteria/toxins

Figure 6.2 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Figure 6.5 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

T cell receptor: antigen receptor of T cells

Antigen

Antigen presentation by MHC molecules

- Two types of MHC molecules
  - MHC class I
  - MHC class II
- Two types of T cells
  - CD4 T helper cells
  - CD8 T cytotoxic cells
- Two types of antigen
  - Endogenous (from within the cell)
  - Exogenous (from outside the cell)

MHC Class I Pathway

MHC Class II Pathway

Peptide within groove

8-10 amino acids

15-20 amino acids

Peptides extends beyond groove

Type of T cell activated

- MHC class I activate CD8+ T cytotoxic cells
- Present endogenous (eg viral) antigens
- Target cell is killed by CD8+ T cell
- Any cell can become virally infected

- Therefore: MHC class I is on all nucleated cells

(b)
Peptide–class I MHC complex
Class I MHC
viral peptide
Vesicle
Golgi complex
Polysomes
Viral
peptides
Rough
endoplasmic
reticulum
Viral
protein
Ribosome
Viral mRNA
Viral DNA
Virus

CTL-target cell
conjugate
Granule
Conjugate
formation
CTL
cytoplasmic
rearrangement
CTL
Target cell
CTL granule
exocytosis
CTL recycling
Dissociation

Some Effector Molecules of CTLs

Protein in granules of cytotoxic T cells	Actions on target cells
Perforin	Aids in delivering contents of granules into the cytoplasm of target cell
Granzymes	Serine proteases, which activate apoptosis once in the cytoplasm of the target cell
Granulysin	Has antimicrobial actions and can induce apoptosis

Type of T cell activated

- MHC class II activate CD4 T helper cells
- Present exogenous (eg bacteria) antigens

- CD4 T cells upregulate all immune functions

- MHC class II found only on cells that sample the extracellular environment

MHC class II-CD4 T cell activation

- CD4 T helper cells up regulate immune function
- Stimulate Mϕ activation and phagocytosis, B cell antibody production
- Help to clear exogenous antigen (ie. parasites)

(a)
Peptide–class II
MHC complex
Antigen ingested
by endocytosis
or phagocytosis
Peptides of
antigen
Class II
MHC
Lysosome
Endosome
Endocytic processing pathway
Nucleus

CD4+ T cell

A question of language

- Cells that express MHC class I, present endogenous ag
  - Are killed by CD8+ cells
  - Are called **Target cells**
- Cells that express MHC class II, present exogenous ag
  - Activate the CD4 T cell response
  - Are called **Antigen presenting cells**

- Target cells:
  - All nucleated cells express MHC class I
  - Can be a target cell if virally infected
- Antigen presenting cells
  - Limited number of cells express MHC class II
  - Must sample extracellular environment
  - Monocytes, MΦ, B cells

How does a cell present pathogens, such as viruses that have been engulfed from *extracellular* sources in ways that will activate the needed CTL responses?

Cross-presentation

- *The phenomenon of cross-presentation requires that internalized antigens that would normally be handled by the exogenous pathway leading to class II MHC presentation somehow become redirected to a class I peptide loading pathway*.

Cross Presentation

1. Cytosolic route
2. Vacuolar route

Figure 6.13 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Cells involved in cross-presentation include
Dendritic and macrophages.

Read on DC licensing in the activation of CTLs

Read a review from Kurts et al 2010 Nature Immunology Review

(a) DC licensing by T_H cell

(b) DC cross-presentation and activation of CTL

Antigen presentation: display of cell's proteome on MHC molecules for T cell recognition

Dendritic cells activate T cells in an antigen-specific manner: Three-signal model

Signal 0: DCs and T cells come together
Signal 1: Ag-specific T cell activation
Signal 2: T cell survival
Signal 3: T cell differentiation

It takes 3 not 2!!!

Figure 9.19 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Applying The Brakes

Figure 9.22 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

We never walk alone: LFC

Figure 9.23 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Role of coceptors in TCR signalling

Intracellular signalling Pathway

Functional Activities of CD4+ T cells

Figure 9.28 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Effector T cell Differentiation

Transcriptional Factors

Figure 9.29 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

EXOGENOUS PATHWAY
ENDOGENOUS PATHWAY
Extracellular bacteria, parasites, toxins
Intracellular bacteria, viruses, tumors
Internalization by APCs
Infection or tumorigenesis
Autophagy
Antigen degradation in endocytic compartment
Antigen degradation in cytosol
Cross-presentation
Peptide association with MHC class II
Peptide association with MHC class I
Activation of CD4+ Th cells
Activation of CD8+ Tc cells

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Intracellular Signalling Pathway

Functional Activities of CD4+ T cells

Figure 9.28 Janeway's Immunobiology, 8ed. (© Garland Science 2012)

Effector T cell Differentiation

Transcriptional Factors

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EXOGENOUS PATHWAY
ENDOGENOUS PATHWAY
Extracellular bacteria, parasites, toxins
Intracellular bacteria, viruses, tumors
Internalization by APCs
Infection or tumorigenesis
Autophagy
Antigen degradation in endocytic compartment
Antigen degradation in cytosol
Cross-presentation
Peptide association with MHC class II
Peptide association with MHC class I
Activation of CD4+ Th cells
Activation of CD8+ Tc cells

T & B Cell Development

γδ
HSC
CMP
GMP
MEP
PMN
Mo
RBC
Mk
Platelets
MPP-ELP
CLP
Pre-proB
ProB
PreB
IgM
IgD
B-2
B-1
ETP
DN2
DN3
DN4
CD4+CD8+
CD4+
CD8+
Embryo
Neonate
Young adult
Old adult

Word/Terms List

Activation
Differentiation
Double negative cells
Double positive cells
Effector cells
Maturation
Negative selection
Positive selection

Lymphopoiesis

- T cell progenitors originate in the BM (~50million per day)
- Migrate to thymus
- Characteristic surface marker and genetic/intracellular changes
- 90% never make it to maturity, i.e. only 10% do
- Apoptosis hits those that do not have functional TCR or don’t get ”selected”

Lymphopoiesis

- T cell generation slows down with age
- Mature T cells may divide in secondary lymphoid organs
- What is consequence of that?

T Cell Maturation

- Hematopoietic stem cells(HSC)
- Lymphoid stem cell (progenitor)
- Circulating lymphoid stem cells
- Thymocytes

T cells develop in the thymus

T cells (T-lymphocytes) = thymus-dependent lymphocytes

Figure 5-1 The Immune System, 2/e (© Garland Science 2005)

The thymus contains

- Thymocytes (immature T cells)
- Thymic stroma (epithelial cells)

- The thymus is a primary lymphoid organ because it is only involved in development, not fighting infection

Figure 5-2 The Immune System, 2/e (© Garland Science 2005)

The Thymus

capsule
trabeculae
Cortex
sub-
capsular
epithelium
cortico-
medullary
junction
Medulla
Hassall's
corpuscle
cortical
epithelial cell
(thymicorigin)
thymocyte
(bone marrow
origin)
medullary
epithelial cell
(thymicorigin)
dendritic cell
(bone marrow
origin)
macrophage
(bone marrow
origin)

Two areas of the thymus

- **Cortex** – outer, close-packed consists of ectodermal cells; can contain thymocytes and macrophages
- **Medulla** – inner, less dense consists of endodermal cells; contains thymocytes, dendritic cells, and macrophages

- **Thymic anlage**: The combination of the ectodermal and endodermal cells, colonized by progenitor cells from the bone marrow.

Thymus facts

- Fully developed at birth and increases in size until puberty
- Most active in the young
- Degrades after puberty (involution), being replaced with fat tissue
- Even after involution (~30 yrs. old) or a thymectomy immunity by T cells is not impaired significantly
- Mature T cell repertoire is long-lived and self-renewing

T Cell Maturation

- Young thymocyte (T cell precursor)
- Double negative thymocytes
- Double negative with early TCR expression
- Double positive with TCR expression
- Naïve CD4 and CD8 T cells

Surface markers
Marrow
Blood
Thymus
Blood
Peripheral tissues
Hematopoietic stem cell (HSC)
Common lymphoid precursor
c-Kit
CD25
CD44
migration
T-cell precursor
TCR locus rearrangement
Dβ-Jβ
Pro-T cell (double negative, DN)
RAG expression on
Vβ-Dβ-Jβ
Pre-T cell (double negative, DN)
CD3
TCR β chain
Pre-Tα
Vβ-Dβ-Jβ and Vβ-Jβ
Pro-T cell (double positive, DP)
TCR α chain
CD4 and CD8
CD4 or CD8
CD8+
CD4+
Tc cell
migration
CD8+
CD4+

Thymic Selection

Positive selection

- Double positives bind MHC molecules
- Nonbinders die
- Binders become single positives

Negative selection

- CD4 or CD8 cells that survive positive selection may react or bind to self MHC alone with high affinity or with Self MHC-self Ag complexes
- These cells are programmed to die

- Nonbinders survive

T-cell precursor
Rearrangement of TCR genes
CD8
CD3
T-cell receptor
Immature
thymocyte
CD4
Positive selection of
cells whose receptor
binds MHC molecules
Death by apoptosis
of cells that do not interact
with MHC molecules
Class I and/or class II
MHC molecules
Epithelial
cell

Class I and/or class II
MHC molecules
Epithelial
cell
Negative selection and death of
cells with high-affinity receptors
for self-MHC or self-MHC +
self-antigen
CD4+
CD8+
TH cell
TC cell
Mature CD4+ or
CD8+ T lymphocytes
Macrophage
Dendritic cell

B Cell Production and development

- Origin…
- Development…
- Selection**S**
- Activation**S**

- Progenitor B cells (pro-B-cells) bearing a CD45R marker develop – in the bone marrow – into immature and then mature B cells.
- Then, they migrate to peripheral lymph nodes.
- Those developmental events are physically and physiologically distinct; the former are antigen *in*dependent; the latter are antigen *de*pendent.

ANTIGEN-INDEPENDENT PHASE
(maturation)
Progenitor
B cell
Ig-gene
rearrangement
Selection
Mature
B cell
CD45R
(B220)
surface
marker
Bone marrow
~5 × 10^6 per day
ANTIGEN-DEPENDENT PHASE
(activation and differentiation)
Naive
B cell
-Ag
Cell death
(~90%)
T_H cell
+Ag
(~10%)
Affinity
maturation
Class
switching
Memory
B cell
Plasma
cell
Secreted
Ab
Peripheral lymphoid organ

Immature B cells
Pre-B cells
Pro-B cells
IL-7
receptor
IL-7
c-Kit
SCF
VLA-4
VCAM-1
mIgM
Bone-marrow
stromal cell

Pro-B cell
Pre-B cell
Immature B cell
Ig-α/Ig-β
$V_HD_HJ_HC_\mu$
Surrogate light chain
Vpre-B
λ5
$V_HD_HJ_HC_\mu$
κ or λ
Crosslinking by stromal-cell ligand
Crosslinking by antigen
Activation
Death
Stops $V_H \rightarrow D_HJ_H$ (allelic exclusion) ?
Induces $V_\kappa \rightarrow J_\kappa$?

CD45R
(B220)
surface
marker
D_H J_H
Surrogate light
chain of pre-BCR
V_H D_HJ_H
IgM
V_L J_L
IgM
IgD
PRO-B CELL
PRE-B CELL
IMMATURE B CELL
MATURE B CELL

Cytokines of various types are secreted by T-helper cell and are received by differentiating B-cells

The various cytokines affect what immunoglobulin isotypes will be produced by plasma cells

Plasma cells
Proliferating B cells
(centrocytes)
IFN-γ
TGF-β
IgG2a or
IgG3
IgA or
IgG2b
IL-2,
IL-4,
IL-5
IL-4
IgE or
IgG1
IL-2,
IL-4,
IL-5
IgM
Activated B cell
(centroblast)
Proliferation cytokines:
IL-2, IL-4, IL-5
Differention cytokines:
IL-2, IL-4, IL-5, IFN-γ, TGF-β

Where is activation happening? And what is happening?

- The primary response is initiated in the paracortex.
- T-cells respond to processed antigen that is typically delivered from a site of origin by dendritic cells.
- B-cells see the same antigen in an unprocessed form in the lymph

Primary follicle
B-cell activation
Afferent lymphatic vessels
B
Paracortex
Initial T-cell and B-cell activation
Germinal center
B-cell proliferation and differentiation
T
B+T
Cortex
Secondary follicle
Medulla
Efferent lymphatic vessel
Plasma-cell secretion of antibody

- 1. Any substance which is capable of inducing an immune response is referred to as an antigen
- a) True b) False
- 2.The transcriptional factor for Th1 cells is called……..
- 3. The signature molecule of Th17 response is……..
- 4. Which of the following cells produce antibodies?
- QUIZ 2 T cells b) dendritic cells c) monocytes d) plasma cells
- 5. Interleukins are proteins which help cell-cell communication
- True b) False
- 6. Name the five main classes of Immunoglobulins and give the subclasses of IgG.
- 7. T cells are produced in the ……..and mature in the ……
- 8. Give 2 features of an immunogen
- 9. Which cytokine help the differentiation of plasma cells into IgE?
- 10. Granzymes are normally (percentage wise) produced by activated CD8+ T cells. What is the function of this molecule?

Immunological Techniques

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Objectives

- At the end of this lecture students should be able to understand the principles underlying common immunological techniques such as FACS and ELISA
- The application of these techniques will be discussed

Flow cytometry is a technique for measuring physical and chemical properties of individual cells as they travel in suspension one by one past a sensing point.

BASICS OF FLOW CYTOMETRY

- The cells in suspension are forced to pass in a fluid stream through a flow cell.
- The fluid stream intersects the focus of a laser.
- The laser light is scattered and, if the cells are fluorescent, they produce fluorescent signals.
- These light signals are then converted to electronic signals (voltages).

INSTRUMENTATION

1. Fluidics
2. Optics
3. Electronics

FLUIDICS

- The purpose of the fluidics system is to transport cells in a fluid stream to the laser beam for interrogation.

OPTICS

The optics system consist of a laser to illuminate the cells in the sample stream and optical filters to direct the resulting light signals to the appropriate detectors (i.e . to resolve different colors).

1. The flow of sheath fluid restricts the cells to the center of the sample core for optimal illumination (hydrodynamic focusing).

2. Only one cell should move through the laser beam at a given moment.

“THE LASER”

- Lasers emit coherent light, in a fine, straight beam at a specified wavelength.
- The use of a laser allows the beam of light to be focused on single cells so that basic measurements based on beam disturbance can be taken (FSC, SSC) .

LASER: Acronym for Light Amplification by Stimulated Emission of Radiation

ELECTRONICS

- Processing of signals from detectors

  -Cells passing through the laser beam generate light signals. These light signals are then converted to electronic signals (voltages).

  -The electrical voltage generated will be proportional to the number of photons (amount of light) emitted by the cell/particle.

  -The voltages are processed by the computer.

The sample is injected into a stream of sheath fluid within the flow chamber; the sample core remains separate but coaxial within the sheath fluid.

- Light is bent (diffracted) depending on the size and refractive index of the cell
- Detected along the axis of incident light ($0\text{-}10^0$)

- Light is reflected/bounced to the side
- Proportional to cell granularity
- Detected at 90° to incident light axis

When cells pass through the laser intercept, they scatter laser light and the system measures the degree and direction of scattered light-indicators of the cell's size, shape and structure.

FACS
Academy

What do the scatter signals tell us?

- Together the forward and side scatter signals can provide useful ways to characterize different cell types.
  - Example: Leucocytes (white blood cells).

SSC-H
FSC-H
0
50
100
150
200
250
neutrophils
monocytes
lymphocytes
lysed whole blood

FLUOROSCENCE

- Fluorescent compounds absorb light energy over a range of wavelengths, which causes an electron in the compound to be raised to a higher energy level.
- The excited electron emits this excess energy as a photon of light.
- While each fluorochrome will have an optimal, or peak emission wavelength, the spectra will actually be distributed over a number of wavelengths.

Any fluorescent molecules present on the cell fluoresce.

- **The cells are forced to pass in a fluid stream through a flow cell where the fluid stream intersects the focus of the laser.**

Detector	Fluorochrome	Emission
FL1	FITC, GFP, Alexa 488, CFSE, Fluo 3	525 nm
FL2	PE, PI	575 nm
FL3	PER-CP, Cychrome, 7-AAD	660-675 nm

More than one fluorochrome can be used simultaneously if each is excited at 488 nm and if the peak emission wavelengths are not extremely close to each other.

DATA DISPLAY

Negative Control

Sample

Histogram: Relative fluorescence vs. # of events

DATA DISPLAY

Quadrants can be applied to any 2 parameter display.

DATA DISPLAY

APOPTOSIS

FasL
TNF-α
Fas
TNFR-1
RIP/RAIDD
FADD
Caspase-8
Bid
Cytochrome C
Caspase
Activation

METHODS FOR DETECTING APOPTOSIS: Annexin V assay

ADVANTAGES

Evaluating large number of cells quantitatively and reproducibly increases the statistical confidence and precision of data.

-The ability to measure several parameters on thousands of single cells within minute (10,000 cells/second).

-Measurements are made separately on each cell.

-Simultaneous, multi-parameter analyses in complex cell populations.

The power of flow cytometry lies in the ability to measure several parameters on thousands of single cells within minutes
(1000 cells/second).

BD FACScan is a 3-colour, fixed alignment bench top analyzer, equipped with a 488 nm air-cooled argon laser. It is capable of simultaneously measuring and analyzing FSC, SSC, and 3 spectral regions of fluorescence.

Frequency of CD8+GZMB+ expressing T cells in persons with filarial infection

Enzyme Linked Immunosorbent Assay (ELISA)

ELISA

- Enzyme Linked Immunosorbent Assay (ELISA)
- Term Was Coined By Engvall and Pearlmann in 1971
- Different Types
  - Sandwich
  - Indirect
  - Competitive
- Can Be Used To Detect Both Antibody and Antigen
- Very Sensitive, pg/mL
- Relies on Monoclonal Abs

- ELISA also has commercial applications;
- including the detection of disease markers and allergens in the diagnostic and food industries.

icroplate reader

ELISA micr
washer

(a) Indirect ELISA
wash
wash
wash
E
S
Antigen-coated well
Add specific antibody to be measured
Add enzyme-conjugated secondary antibody
Add substrate (S) and measure color
(b) Sandwich ELISA
wash
wash
wash
E
S
Antibody-coated well
Add antigen to be measured
Add enzyme-conjugated secondary antibody
Add substrate and measure color
(c) Competitive ELISA
wash
wash
E
S
Incubate antibody with antigen to be measured
Add Ag-Ab mixture to antigen-coated well
Add enzyme-conjugated secondary antibody
Add substrate and measure color

Sandwich ELISA

- 2 Antibodies Required
- Must Recognize Different Epitopes
- 1st Antibody Is Referred To As Capture Ab
- 2nd Antibody Detection Ab
- 2nd Antibody Is Biotinylated
- Enzymes Commonly Used: HRP (Horse Radish Peroxidase) And AKP (Alkaline Phosphatase)
- Substrate is TMB (Chromogen)

ELISA Plate

- 96 well plate
- Made of plastic on which protein can be adsorbed (bind) easily
- Usually done overnight @ 4°C
- Special buffer used that will not denature Ab and maximize binding
- Blocking step ensures no empty spaces are left
- Blocking reagent is often 10% FBS

96 well plate

Multi-channel pi

Standard Curve

- Serial dilutions of the cytokine being measured
- Exact concentration is needed
- A plot of concentration (pg/mL or ng/mL) is plotted against OD (optical density)

Sensitivity Of Elisa

- Typically, the lowest cytokine concentration that can be detected above negative control
- 2-3 S.D Above Mean Background Signal
- Depending On Antibody Pair Used Sensitivity Varies
- Ex. 10 pg/mL

Blocking

- Blocking Reagent 10% FBS in PBS
- Alternatively, 1% BSA (Immunoassay Grade)
- Why Do We Block?

After Blocking

- Wash x3 With PBS/Tween (detergent)
- Add Standards + Samples
- Samples Are Typically Supernatants From Cultures Or Patient Serum/Plasma
- Use 100 μL
- Often Dilution Is Required If Signal Is Too Strong
- Standards?

Standard Preparation

- Standards Are Diluted in Blocking Buffer/Tween
- Start By Labeling eight, 1 mL Eppendorf Tubes
- Prepare Highest Conc. Tube (1 mL)
- Fill The Remaining Tubes with 0.5 mL Blocking Buffer
- Serially Dilute From Top To Lowest

Serial Dilution

After Standard Preparation

- Add Samples, Standards, Negative Control
  - Negative Control Should Be The Buffer You Use Dilute Standard or Culture Medium
- Incubate For 2 Hrs at R.T
- Aspirate And Wash 5x

(a) Indirect ELISA
wash
wash
wash
Antigen-coated well
Add specific antibody to be measured
Add enzyme-conjugated secondary antibody
Add substrate (S) and measure color
(b) Sandwich ELISA
wash
wash
wash
Antibody-coated well
Add antigen to be measured
Add enzyme-conjugated secondary antibody
Add substrate and measure color
(c) Competitive ELISA
wash
wash
Incubate antibody with antigen to be measured
Add Ag-Ab mixture to antigen-coated well
Add enzyme-conjugated secondary antibody
Add substrate and measure color

Addition Of Detection Ab

- Avidin has very high affinity for biotin (B vitamin)
- Add Working Detector @ 100 μL/well
- Incubate for 60 mins @ R.T
- Wash 3x

Addition of Substrate

- Add Substrate according to protocol
- Add 100 μL/well
- Incubate for 10 mins,
- Terminate Reaction by Adding 0.5 M H_2SO_4 (color changes from blue to yellow)

Read Plate At Appropriate Wavelength (λ=450 nm)

10
8
6
4
2
0
TNF-α (ng/mL)
Medium
10
1
0.1
0.01
NS398 μM
LPS (1 μg/mL)