Biochemistry of the immune system

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The innate immune system - Introduction

• An antigen-nonspecific defense mechanisms

• Used immediately or within several hours after exposure to almost any microbe.

• This is the immunity one is born with and is the initial response by the body to eliminate microbes and prevent infection.

• Innate immunity can be divided into:
  – immediate innate immunity
  – early induced innate immunity.
The innate immune system – Cells in Blood

• Polymorphonuclear leukocytes (granulocytes)
  – Neutrophils
  – Eosinophils
  – Basophils

• Mononuclear leukocytes (agranulocytes)
  – Monocytes and macrophages
    • Phagocytes
    • Differentiate into macrophages and dendritic cells
    • Serve as antigen-presenting cells in the adaptive immune responses
    • Cytokines production

  – Lymphocytes (adaptive immune responses)
    • NK cells
      – kill cells to which antibody molecules have attached - antibody-dependent cellular cytotoxicity (ADCC)
      – kill human cells lacking MHC-I molecules on the surface
The innate immune system – Cells in Tissues

• **Dendritic Cells**
  – In the epithelium of the skin, the respiratory tract, the gastrointestinal tract, lymphoid tissues and organ parenchyma
  – capture and present protein antigens to naive (unused) T-lymphocytes

• **Fixed macrophages**
  – part of the mononuclear reticuloendothelial system
  – Kill microbes, infected cells, and tumor cells by phagocytosis
  – Process antigens so they can be recognized by effector T-lymphocytes
  – Secret lipid mediators of inflammation such as leukotrienes, prostaglandins, and platelet-activating factor (PAF)
  – Cytokines

• **Mast Cells**
  – In connective tissue or mucous membranes
  – Pattern-recognition receptors or PRRs on the surface that interact with pathogen-associated molecular patterns or PAMPs of microbes. After the PAMPs bind to their respective PRRs, they release the contents of their granules. These chemical mediators promote inflammation and attract neutrophils to the infected site.
  – histamine, eosinophil chemotactict factor, neutrophil chemotactict factor, platelet activating factor, and cytokines such as IL-3, IL-4, IL-5, IL-6, and TNF-alpha. Synthesis of leukotrienes, prostaglandins, chemicals that promote inflammation by causing vasodilation, increasing capillary permeability, and increasing mucous production
Immediate Innate Immunity
(0-4 hours after exposure to an infectious agent)

• Antimicrobial enzymes and Antimicrobial Peptides

• The Complement System
  – The Classical Complement Pathway
  – The Lectin Pathway
  – The Alternative Complement Pathway

• Anatomical Barriers to Infection. Mechanical Removal of Microbes. Bacterial Antagonism by Normal Body Microbiota.
Antimicrobial enzymes and Antimicrobial Peptides

• **Lysozyme**
  – breaks the bond between the N-acetylglucosamine and N-acetylmuramic acid in peptidoglycan of bacterial cells

• **Phospholipase A\textsubscript{2}**
  – hydrolyzes the phospholipids in the bacterial cytoplasmic membrane

• **Human defensins** - cationic peptides 30-40 amino acids long
  – directly toxic by disrupting the cytoplasmic membrane of a variety of microorganisms
  – activate cells for an inflammatory response
  – disrupt the envelopes of some viruses
Antimicrobial enzymes and Antimicrobial Peptides

• Cathelicidins - proteins produced by skin and mucosal epithelial cells
  – directly toxic to a variety of microorganisms
  – bind to and neutralize LPS from Gram-negative cell walls

• Lactic and fatty acids
  – inhibit microbes on the skin

• Lactoferrin and transferrin
  – trap iron for use by human cells while preventing its use by microorganisms

• Hydrochloric acid
  – destroy microbes that are swallowed

• **Anatomical barriers**
  – The skin
  – The mucous membranes
  – Bony encasements

• **Mechanical removal**
  – Mucus and cilia
  – The cough and sneeze reflex
  – Vomiting and diarrhea
  – The physical flushing action of body fluids

• **Bacterial Antagonism by Normal Microbiota**
  – Producing metabolic products (fatty acids, bacteriocins, etc.) that inhibit the growth of many pathogens
  – Adhering to target host cells so as to cover them and preventing pathogens from colonizing
  – Depleting nutrients
  – Non-specifically stimulating the immune system.
Early Induced Innate Immunity

- Pathogen-Associated Molecular Patterns (PAMPs) and Danger-Associated Molecular Patterns (DAMPs)
- Pattern-Recognition Receptors (PRRs)
- Cytokines Important in Innate Immunity
- Phagocytosis
- Natural Killer Cells (NK Cells) and Invariant Natural Killer T-Lymphocytes (iNKT Cells)
- Inflammation
- Nutritional Immunity
- Fever
- The Acute Phase Response
- Intraepithelial T-lymphocytes and B-1 cells
Pathogen-Associated Molecular Patterns (PAMPs) and Danger-Associated Molecular Patterns (DAMPs)

- **Microbial-associated PAMPs**
  - lipopolysaccharide (LPS) from the outer membrane of the Gram-negative cell wall
  - bacterial lipoproteins and lipopeptides
  - porins in the outer membrane of the Gram-negative cell wall
  - peptidoglycan found abundantly in the Gram-positive cell wall and to a lesser degree in the gram-negative cell wall
  - lipoteichoic acids found in the Gram-positive cell wall
  - lipoarabinomannan and mycolic acids found in acid-fast cell walls
  - mannose-rich glycans (short carbohydrate chains with the sugar mannose or fructose as the terminal sugar). These are common in microbial glycoproteins and glycolipids but rare in those of humans
  - flagellin found in bacterial flagella;
  - bacterial and viral nucleic acid. Bacterial and viral genomes contain a high frequency of unmethylated cytosine-guanine dinucleotide or CpG sequences (a cytosine lacking a methyl or CH3 group and located adjacent to a guanine). Mammalian DNA has a low frequency of CpG sequences and most are methylated which may mask recognition by pattern-recognition receptors. Also, human DNA and RNA does not normally enter cellular endosomes where the pattern-recognition receptors for microbial DNA and RNA are located
  - N-formylmethionine, an amino acid common to bacterial proteins
  - double-stranded viral RNA unique to many viruses in some stage of their replication
  - single-stranded viral RNA from many viruses having an RNA genome
  - lipoteichoic acids, glycolipids, and zymosan from yeast cell walls
  - phosphorylcholine and other lipids common to microbial membranes.
Pathogen-Associated Molecular Patterns (PAMPs) and Danger-Associated Molecular Patterns (DAMPs)

- DAMPs associated with stressed, injured, infected, or transformed host cells and not found on normal cells
  - heat-shock proteins
  - altered membrane phospholipids
  - molecules normally located inside phagosomes and lysosomes that enter the cytosol only when these membrane-bound compartments are damaged as a result of infection, including antibodies bound to microbes from opsonization.
  - molecules normally found within cells, such as ATP, DNA, and RNA, that spill out of damaged cells.
Pattern-Recognition Receptors (PRRs) and Danger-Recognition Receptors (DRRs)

• Endocytic (Phagocytic) Pattern-Recognition Receptors
  – Mannose receptors
  – Dectin-1
  – Scavenger receptors
  – Opsonin receptors
  – N-formyl Met receptors
Pattern-Recognition Receptors (PRRs) and Danger-Recognition Receptors (DRRs)

- Signaling Pattern-Recognition Receptors
  
  - Signaling PRRs found on cell surfaces - toll-like receptors (TLRs), CD14
  - Signaling PRRs found in the membranes of the endosomes (phagolysosomes) – binds viral RNA and unmethylated cytosine-guanine dinucleotide sequences (CpG DNA)
  - Signaling PRRs and DRRs found in the cytoplasm - NODs (nucleotide-binding oligomerization domain)
Cytokines Important in Innate Immunity

- **Pleiotropic** means that a particular cytokine can act on a number of different types of cells rather than a single cell type.
- **Redundant** refers to the ability of a number of different cytokines to carry out the same function.
- **Multifunctional** means the same cytokine is able to regulate a number of different functions.

- Tumor necrosis factor-alpha (TNF-α) - mediates acute inflammation
- Interleukin-1 (IL-1) - mediates acute inflammation
- Chemokines - enable the migration of leukocytes from the blood to the tissues
- Interleukin-12 (IL-12) - stimulate the synthesis of interferon-gamma by T-lymphocytes, stimulates the differentiation of naive T4-lymphocytes
- Type I Interferons - all components of the immune system
- Interleukin-6 (IL-6) - stimulate the liver to produce acute phase proteins
- Interleukin-10 (IL-10) - inhibitor of activated macrophages and dendritic cells
- Interleukin 15 (IL-15) - stimulates NK cell proliferation and proliferation of memory T8-lymphocytes
- Interleukin-18 (IL-18) - stimulates the production of interferon-gamma by NK cells and T-lymphocytes
Phagocytosis

• neutrophils, eosinophils, and monocytes; tissue phagocytic cells in the tissue such as macrophages
Natural Killer Cells (NK Cells) and Invariant Natural Killer T-Lymphocytes (iNKT Cells)

• Natural Killer Cells (NK Cells)

  – **NK cells** are important in innate immunity because they are able to **recognize infected cells, cancer cells, and stressed cells and kill them**. In addition, they produce a variety of cytokines, including proinflammatory cytokines, chemokines, colony-stimulating factors, and other cytokines that function as regulators of body defenses. For example, through cytokine production NK cells also suppress and/or activate macrophages, suppress and/or activate the antigen-presenting capabilities of dendritic cells, and suppress and/or activate T-lymphocyte responses.

  – **NK cells** use a **dual receptor system** in determining whether to kill or not kill human cells. When cells are either under stress, are turning into tumors, or are infected, various stress-induced molecules such as MHC class I polypeptide-related sequence A (MICA) and MHC class I polypeptide-related sequence B (MICB) are produced and are put on the surface of that cell.
Natural Killer Cells (NK Cells) and Invariant Natural Killer T-Lymphocytes (iNKT Cells)

- **Invariant Natural Killer T-Lymphocytes (iNKT Cells)**

  - **iNKT cells** are a subset of lymphocytes that bridge the gap between innate and adaptive immunity. They have **T-cell receptors** (TCRs) on their surface for glycolipid antigen recognition. They also have **natural killer (NK) cell receptors**.

  - Through the cytokines they produce once activated, **iNKT cells are essential in both innate and adaptive immune protection** against pathogens and tumors. They also play a regulatory role in the development of autoimmune diseases, asthma, and transplantation tolerance. It has been shown that iNKT cell deficiency or disfunction can lead to the development of autoimmune diseases, human asthma, and cancers.

  - **Pathogens may not directly activate iNKT cells.** The TCR of iNKT cells recognize exogenous glycolipid antigens, as well as endogenous self glycolipid antigens presented by MHC-I-like CD1d molecules on antigen presenting dendritic cells. iNKT cells can also be activated by the cytokine interleukin-12 (IL-12) produced by dendritic cells that have themselves become activated by pathogen-associated molecular patterns (PAMPs) of microbes binding to the pattern-recognition receptors (PRRs) of the dendritic cell.

  - Once activated, the iNKT cells rapidly produce large quantities of cytokines, including interferon-gamma (IFN-γ), interleukin-4 (IL-4), interleukin-2 (IL-2), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), interleukin-13 (IL-13), and chemokines. Through the rapid productions of such cytokines, iNKT cells are able to promote and suppress different innate and adaptive immune responses. For example, large amounts of IFN-γ are produced by activated iNKT cells. IFN-γ activates NK cells and macrophages as a part of innate immunity.
Inflammation

- **Smooth muscles** around larger blood vessels **contract** to **slow the flow of blood** through the capillary beds at the infected or injured site.
- **Vasodilatation**
- **Selectins** are produced on the membrane of the leukocyte and are able to reversibly bind to corresponding **selectin glycoprotein receptors** on the inner wall of the venule. This reversible binding enables the leukocyte to roll along the inner wall of the venule. Adhesion molecules are activated on the surface of the endothelial cells on the inner wall of the capillaries. Corresponding **molecules on the surface of leukocytes** called **integrins** attach to these adhesion molecules allowing the leukocytes to flatten and squeeze through the space between the endothelial cells. This process is called **diapedesis** or **extravasation**.
- **Activation of the coagulation pathway** causes fibrin clots to physically trap the infectious microbes and prevent their entry into the bloodstream. This also triggers blood clotting within the surrounding small blood vessels to both stop bleeding and further prevent the microorganisms from entering the bloodstream.
Nutritional Immunity

- Leukocyte-endogenous mediator (LEM)
  - decreased intestinal absorption of iron from the diet
  - decrease of iron in the plasma and an increase in iron in storage as ferritin
  - increased synthesis of the human iron-binding proteins (iron chelators) such as lactoferrin, transferrin, ferritin, and hemin that trap iron for use by human cells while making it unavailable to most microbes
  - coupled with the febrile response, decreased ability of bacteria to synthesize their own iron chelators called siderophores;
  - prior stationing of lactoferrin at common sites of microbial invasion such as in the mucous of mucous membranes, and the entry of transferrin into the tissue during inflammation.
Fever

• Fever increases the environmental temperature above the optimum growth temperature for many microorganisms. If the microorganisms are growing more slowly, the body's defenses have a better chance of removing them all.

• Fever leads to the production of heat shock proteins that are recognized by some intraepithelial T-lymphocytes called delta gamma T-cells, resulting in the production of inflammation-promoting cytokines.

• Fever elevates the temperature of the body increasing the rate of enzyme reactions, and speeding up metabolism within the body. An elevation in the rate of metabolism can increase the production and activity of phagocytes, speed up the multiplication of lymphocytes, increase the rate of antibody and cytokine production, increase the rate at which leukocytes are released from the bone marrow into the bloodstream, and speed up tissue repair.
The Acute Phase Response

- **Activated macrophages** and other leukocytes **release inflammatory cytokines** such as tumor necrosis factor-alpha (TNF-alpha), **interleukin-1** (IL-1), and **interleukin-6** (IL-6) when their pattern-recognition receptors (PRRs) bind pathogen associated molecular patterns or PAMPs.

  - **C-reactive protein (CRP)** binds to the phosphorylcholine portion of teichoic acids and lipopolysaccharides of bacterial and fungal cell walls. It also binds to the phosphorylcholine found on the surface of damaged or dead human cells. It functions as an **opsonin**, sticking the microorganism to phagocytes, and activates the classical complement pathway by binding **C1q**, the first component in the pathway.

  - **Mannan-binding lectin (MBL)** - also known as mannan-binding protein or MBP - binds to mannose-rich glycan chains with the sugar mannose or fructose as the terminal sugar). These are **common in microbial glycoproteins and glycolipids** but rare in those of humans. It functions as an **opsonin**, sticking the microorganism to phagocytes, and activates the lectin pathway.
Intraepithelial T-lymphocytes and B-1 cells

• Subpopulations of T-lymphocytes and B-lymphocytes that possess a more limited diversity of receptors and are designed to **directly recognize the more common microbes** that enter the epidermis or the mucosal epithelia. As such, they function more as effector cells for innate immunity rather than adaptive immunity.

  – **Intraepithelial T-lymphocytes** (IELs) are found in the epidermis of the skin and the mucosal epithelia

  – **B-1 lymphocytes**, or B-1 cells are found mostly in the peritoneal and pleural cavities. B-1 cells have a limited diversity of antigen receptors that initially produce a class of antibody molecule called **IgM** against common polysaccharide and lipid antigens of microbes and against PAMPs
Adaptive immunity

• Introduction
• Humoral Immunity
• Cell-Mediated Immunity
Adaptive immunity

- Adaptive (acquired) immunity refers to antigen-specific defense mechanisms that take several days to become protective and are designed to react with and remove a specific antigen. This is the immunity one develops throughout life.

  - Humoral immunity
  - Cell-mediated immunity
Humoral Immunity

- **Humoral Immunity** refers to the production of antibody molecules in response to an antigen. These antibody molecules circulate in the plasma of the blood and enter tissue and organs via the inflammatory response. Humoral immunity is most effective microbes or their toxins located in the extracellular spaces of the body.

- Antibodies or immunoglobulins are specific glycoprotein configurations produced by B–lymphocytes and plasma cells in response to a specific antigen and capable of reacting with that antigen.
Immunoglobulin structure

4 polypeptide chains:
  2 identical heavy (H)
  2 identical light (L)

**H chains:** μ, δ, γ, α, ε
(different amino acid composition)

**L chains:** κ, λ

**Izotypes** = classes of IgG:
IgM (μ)
IgD (δ)
IgG (γ)
IgA (α)
IgE (ε)
Immunoglobulin classes
Primary and secondary antibody response

Primary antibody response:
- First infection
- Antibody-secreting cells in peripheral lymphoid tissues
- Activated B cells
- Native B cell
- Long-lived plasma cells in bone marrow
- Low-level antibody production
- Days after antigen exposure: 0, 7, >30

Secondary antibody response:
- Repeat infection
- Antibody-secreting cells
- IgG
- IgM
- Long-lived plasma cells in bone marrow
- Memory B cell
- Days after antigen exposure: 0, 3, 10, >30

Days after antigen exposure: 0, 7, >30
Antibodies in Body Defence

• Opsonization
• Cytolysis by the Membrane Attack Complex (MAC)
• Antibody-dependent Cellular Cytotoxicity (ADCC) by NK Cells
• Neutralization of Exotoxins
• Neutralization of Viruses
• Preventing Bacterial Adherence
• Agglutination of Microorganisms
• Immobilization of Bacteria and Protozoans
• Promoting an Inflammatory Response
Cell-Mediated Immunity

• Cell-mediated immunity (CMI) is an immune response that does not involve antibodies but rather involves the activation of macrophages and NK-cells, the production of antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen. Cellular immunity protects the body by:

  – Activating antigen-specific cytotoxic T-lymphocytes (CTLs) that are able to destroy body cells displaying epitopes of foreign antigen on their surface, such as virus-infected cells, cells with intracellular bacteria, and cancer cells displaying tumor antigens
  – Activating macrophages and NK cells, enabling them to destroy intracellular pathogens
  – Stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.

• Cell-mediated immunity is directed primarily microbes that survive in phagocytes and microbes that infect non-phagocytic cells. It is most effective in destroying virus-infected cells, intracellular bacteria, and cancers. It also plays a major role in delayed transplant rejection.
Activating Antigen-Specific Cytotoxic T-Lymphocytes (CTLs)

• Marking an Infected Cell or a Tumor Cell for Destruction by Cytotoxic T-Lymphocytes (CTLs)
  – The TCRs and CD8 molecules on the surface of naive T8 lymphocytes are designed to recognize peptide epitopes bound to MHC-I molecules on antigen-presenting cells or APCs
  – b. The TCRs and CD8 molecules on the surface of cytotoxic T-lymphocytes (CTLs) are designed to recognize peptide epitopes bound to MHC-I molecules on infected cells and tumor cells.

• Cytotoxic T-Lymphocyte (CTL) Destruction of Body Cells Displaying Epitopes of Foreign Antigen on their Surface
  – Pore-forming proteins called perforins
  – Proteolytic enzymes called granzymes
  – Granulysin
Activating Macrophages and NK Cells

• Effector T4-lymphocytes called TH1 cells coordinate immunity against intracellular bacteria and promote opsonization by macrophages
  – Increases their production of toxic oxygen radicals, nitric oxide, and hydrolytic lysosomal enzymes enabling the killing of microbes within their phagolysosomes.
  – Causes the macrophages to secrete cytokines such as TNF-α, IL-1, and IL-12. TNF-α and IL-1 promote inflammation to recruit phagocytic leukocytes. IL-12 enables naive T4-lymphocytes to differentiate into TH1 cells.
  – Increases the production of B7 co-stimulator molecules and MHC-1 molecules by macrophages for increased T-lymphocyte activation.

• Cytokines such as interleukin-2 (IL-2) and interferon-gamma (IFN-gamma) produced by TH1 lymphocytes activate NK cells.
  – NK cells kill cells to which antibody molecules have attached through a process called antibody-dependent cellular cytotoxicity (ADCC). The NK cell is then able to contact the cell and by inducing a programmed cell suicide called apoptosis
  – NK cells to use a duel receptor system in determining whether to kill or not kill human cells.
    • killer-activating receptor
    • killer-inhibitory receptor, recognizes MHC-I molecules
Stimulating Cells to Secrete a variety of Cytokines that Influence the Function of Other Cells Involved in Adaptive Immune Responses and Innate Immune Responses
**Overview**

**Innate Versus Adaptive Immunity**

- **Innate Immunity**
  - Antigen-nonspecific
  - Activated by PAMPs binding to PRRs
  - Activated immediately or within several hours after exposure to almost any microbe
  - Examples: Phagocytosis, acute phase response, inflammation, NK cells, fever, lectin complement pathway, anatomical barriers, mechanical removal

- **Adaptive Immunity**
  - Antigen-specific
  - Activated by epitopes of antigens binding to antigen receptors
  - Takes several days to become protective and is designed to react with and remove a specific antigen
  - Memory
  - Examples: Humoral immunity (antibody production), cell-mediated immunity (activated macrophages, cytotoxic T-lymphocytes, CTLs)

- **Immune Responses**
  - Immunity one is born with
  - Immunity one develops throughout life

- **Specificity**
  - PAMP: Pathogen-associated molecular patterns
  - Recognizes molecules shared by groups of related microbes

- **Epitope**
  - The portion of an antigen that reacts with antibodies and B- and T-lymphocyte receptors

- **Branches**
  - Humoral immunity
  - Cell-mediated immunity
Signalling molecules – directly synthesized (Eicosanoids ...)

- compounds containing a 20-carbon core

- Members of this group:
  - prostaglandins
  - prostacyclines
  - tromboxanes
  - leukotrienes
  - lipoxins
  - hydroxyeicosatetraenoic acids (HETE)
  - hepoxilins
Eicosanoids biosynthesis

• A path in metabolism of polyunsaturated fatty acids (PUFAs), mainly linoleic and arachidonic acid arachidonic acid is (in humans) synthesized from linoleic acid:

• !!! it is not possible to synthesise *de novo*

• Most animals cannot form double bonds behind position $\Delta^9 \rightarrow$linoleic and linolenic acids are essential: must be taken from food (plant oils, peanuts, soya beans, maize)
Eicosanoids biosynthesis - overview

Arachidonic acid

- Cyclooxygenase
  - PGG₂
    - Prostaglandins
    - Thromboxanes
  - HPETE
    - Leukotrienes
    - HETE
    - Lipoxins

- Lipoxygenase
  - Epoxides
    - diHETE
    - HETE

- Cytochrome P₄₅₀

HETE
Main eicosanoid production sites

- Endothelial cells
- Leukocytes
- Platelets
- Kidneys

- Unlike e.g. histamin, eicosanoids are not synthesized in advance and stored in granules
- In case of an emergent need, these are rapidly produced from a released arachidonate

- Eicosanoids biosynthesis takes place in every cell type except red blood cells
Main steps of eicosanoids production

1) **Activation of phospholipase A\_2** (PLA2)

2) **Release of arachidonate** into cytosol from membrane phospholipids by PLA2

3) **Eicosanoids synthesis from arachidonate** COX or LO pathway + further modifications by synthases/isomerases (PGH\_2 conversion to other prostanoids, LTA\_4 conversion..) depending on cell type
1) Activation of phospholipase A2

• Ca\(^{2+}\) dependent

• PLA\(_2\) expression / activity stimulate:
  – interleukin-1
  – angiotensin II
  – bradykinin
  – thrombin
  – epinephrine...

• PLA\(_2\) expression / activity block:
  – dexamethasone (synthetic corticoid)
  – annexin 1 (lipocortin) – protein inducible by glucocorticoids
  – caspase-3
2) Arachidonate mobilization for eicosanoid synthesis

• From membrane phospholipids mostly by the action of phospholipase A$_2$:

Release of arachidonate from phospholipids is **blocked** by anti-inflammatory steroids!
Eicosanoids biosynthesis

• 3 pathways:
  – **A)** *cyclooxygenase* – produces prostaglandins and thromboxanes
  – **B)** *lipoxygenase* – produces leukotrienes, lipoxins, hepoxilins and 12- and 15-HETE (hydroxyeicosatetraenoic acids)
  – **C)** *cytochrome P450 enzymes* (monooxygenases) – produces HETE, e.g. 20-HETE; it is a main pathway in kidney proximal tubules
Products of COX pathway
Inhibition of COX pathway

- **Aspirin** inhibits cyclooxygenase activity of PGHS-1 and PGHS-2 (by acetylation of enzyme serine)
- Other **non-steroidal anti-inflammatory drugs** inhibit cyclooxygenase activity (ibuprofen – competes with arachidonate)
- Anti-inflammatory **corticosteroids** block PGHS-2 transcription
- **Corticosteroids**
Lipooxygenase pathway

- 3 different lipoxygenases introduce oxygen to position 5, 12 or 15 in arachidonate; a primary product is hydroperoxy-eicosatetraenoic acid (HPETE)
- Only 5-lipoxygenase produces leukotrienes; it requires protein FLAP

- \[ \text{12-lipoxygenase} \]
- \[ \text{15-lipoxygenase} \]
- \[ \text{5-lipoxygenase} \]

- 12-HPETE → 12-HETE
- 5-HPETE → 5-HETE
- 15-HPETE → 15-HETE
- Leukotriene D₄
- Leukotriene A₄
- Leukotriene C₄
- Leukotriene E₄
- Peptidoleukotrienes

- Gly–Cys–Glu
- Glutathione

- Hepoxilins (HXA₃)
Synthesis of eicosanoids by enzymes CYP<sub>450</sub>

- Cytochrome P450 enzymes – **monooxygenases**:
  \[ \text{RH} + \text{O}_2 + \text{NADPH} + \text{H}^+ \rightarrow \text{ROH} + \text{H}_2\text{O} + \text{NADP}^+ \]

- Two types of compounds are produced:
  - **Epoxygenases** - catalyse production of **epoxyeicosatrienoic acids** (EETs) which are metabolized by epoxid-hydrolases into almost inactive dihydroxyeicosatrienoic acids (DiHETEs)
  - **Hydroxylases** - catalyse production of **HETEs** (20-HETE, 13-HETE etc.)
Eicosanoids - list

- CYP450
  - EETs (epoxides)
    - DiHETEs

- Arachidonic acid
  - Cyclooxygenases
    - Prostacyclins
    - Prostaglandins
    - Tromboxanes
  - Lipoxygenases
    - Leukotrienes
    - Lipoxins
      - 5-, 8-, 12-, 15-HETE
    - Hepoxilins
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