**Introduction**

- Inflammatory response to injury
  - To respond & neutralize microorganisms
  - Coordinate tissue repair
- Major host insult
  - Propagate reactions
  → Systemic inflammation → detrimental response
- Incidence
  - Sepsis 900,000 cases/year
  - Trauma — leading cause under 50 years

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**THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME**

The systemic response to injury
- Proinflammatory phase
  - Activation of cellular processes
  - Restore tissue function
  - Eradicate invading microorganisms
- Anti-inflammatory (counterregulatory) phase
  - Serve to modulate proinflammatory phase
  - Restore homeostasis

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**TABLE 1.1 Clinical Spectrum of Infection and Systemic Inflammatory Response Syndrome (SIRS)**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>Identifiable source of microbial insult</td>
</tr>
<tr>
<td>SIRS</td>
<td>Two or more of following criteria</td>
</tr>
<tr>
<td></td>
<td>Temperature ≤38°C or ≥38°C</td>
</tr>
<tr>
<td></td>
<td>Heart rate ≥90 beats/min</td>
</tr>
<tr>
<td></td>
<td>Respiratory rate ≥20 breaths/min or</td>
</tr>
<tr>
<td></td>
<td>Pao2 ≤50 mm Hg or mechanical ventilation</td>
</tr>
<tr>
<td></td>
<td>White blood cell count ≥12,000 μL or ≤4000 μL or</td>
</tr>
<tr>
<td></td>
<td>≥10% band forms</td>
</tr>
<tr>
<td>Sepsis</td>
<td>Identifiable source of infection + SIRS</td>
</tr>
<tr>
<td>Severe sepsis</td>
<td>Sepsis + organ dysfunction</td>
</tr>
<tr>
<td>Sepsis shock</td>
<td>Sepsis + cardiovascular collapse (requiring vasopressor support)</td>
</tr>
</tbody>
</table>

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**Severe Acute Pancreatitis**

- Week 1 - SIRS / ARDS
  - Early SIRS
  - SIRS / ARDS
- Severe SIRS
  - Severe immune inflammatory response
  - Early SIRS
  - Infections / Late MOD
- Bacterial translocation, sepsis
**CENTRAL NERVOUS SYSTEM REGULATION OF INFLAMMATION**

**Afferent Signals to the Brain**

- CNS respond to stimuli through both the circulatory and neuronal pathways
- Humoral – inflammatory mediators → activate CNS receptors → fever and anorexia i.e. TNF-α
- Neural – vagal sensory input from the site of injury
  - cytokines (e.g., TNF-α and interleukin [IL]-1)
  - baroreceptors
  - chemoreceptors
  - thermoreceptors

**Cholinergic Anti-inflammatory Pathways**

- Parasympathetic nervous system transmits vagus nerve efferent signals through the neurotransmitter Acetylcholine
  - ↓ macrophage activation
  - ↓ macrophage release of pro-inflammatory mediators (TNF-α, IL-1, IL-18) and high mobility group protein (HMG-1)
  - ↓ HR, increases gut motility, dilates arterioles, constricts pupils, and regulates inflammation

**HORMONAL RESPONSE TO INJURY**

**Hormone Signaling Pathways**

- Hormone - chemical classifications
  - polypeptide (cytokine, glucagon, insulin)
  - amino acid (epinephrine, serotonin, histamine)
  - fatty acid (glucocorticoids, prostaglandins, leukotrienes)
- Hormone signal transduction pathways
  - Receptor Kinases – insulin & insulin-like growth factor receptors
  - Guanine nucleotide binding (G-protein) – neurotransmitter and prostaglandin receptors
  - Ligand Gated ion channels

**Quiz**

Regarding Cholinergic Anti-inflammatory Pathways, which is NOT CORRECT?

A. Parasympathetic nervous system activity transmits primarily through the neurotransmitter acetylcholine.
B. This neurally mediated anti-inflammatory pathway allows for a rapid response to inflammatory stimuli, specifically IL-1.
C. Vagus nerve activity in the presence of systemic inflammation may inhibit cytokine activity and reduce injury from disease processes.
D. This activity is primarily mediated through nicotinic acetylcholine receptors on immune mediator cells such as tissue macrophages.
E. Nicotine has been shown to reduce cytokine release after endotoxemia in animal models.
Hormone Signaling Pathways (cont.)

- On activation, signal is amplified through secondary signaling molecules → downstream effects (protein synthesis, mediator release)
- Protein synthesis is mediated through intracellular receptor binding
  - Hormone ligands
  - Secondary signaling molecules
- With targeted DNA sequences, activate transcription

Adrenocorticotropic Hormone

- Synthesized and released by anterior pituitary gland
- Regulated by circadian signals late at night until the hours immediately before sunrise
- Pattern is dramatically altered during injury
  - ↑ corticotropin-releasing hormone and ACTH → proportional to injury severity
- Stimuli for ACTH release: pain, anxiety, vasopressin, angiotensin II, cholecystokinin, vasoactive intestinal polypeptide (VIP), catecholamines, proinflammatory cytokines

TABLE 1-2 Hormones Regulated by the Hypothalamus, Pituitary, and Autonomic System (cont.)

<table>
<thead>
<tr>
<th>Hormones Regulated</th>
<th>Hypothalamic Regulation</th>
<th>Anterior Pituitary Regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticotropin-releasing hormone</td>
<td>Cortisol</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>Thyrotropin-releasing hormone</td>
<td>Somatostatin</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>Growth hormone-releasing hormone</td>
<td>Thyrotropic hormone</td>
<td>Prolactin</td>
</tr>
<tr>
<td>Luteinizing hormone-releasing hormone</td>
<td>Thyroxine</td>
<td>Endorphins</td>
</tr>
</tbody>
</table>

Posterior Pituitary Regulation

<table>
<thead>
<tr>
<th>Vasopressin</th>
<th>Oxytocin</th>
</tr>
</thead>
</table>

Autonomic System

<table>
<thead>
<tr>
<th>Norepinephrine</th>
<th>Epinephrine</th>
<th>Aldosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin-angiotensin system</td>
<td>Insulin</td>
<td>Glucagon</td>
</tr>
</tbody>
</table>

Major Pathway for Adrenal Steroid Synthesis

Cortisol and Glucocorticoids

- Release during stress adrenal cortex in response to ACTH
- Potentiates glucagon & epinephrine → hyperglycemia
  - Liver → ↑ gluconeogenesis ↓ glycogenesis
  - Skeletal m. → protein & amino acid degradation, lactate release
  - Adipose → stimulate release of TG, Free fatty acid, glycerol
- ↑ Transforming growth factor beta (TGF-β) & IGF-1 → Impaired wound healing
Quiz

Regarding Cortisol, which is NOT CORRECT?

A. Stimulates the release of free fatty acids, triglycerides, and glycerol in adipose tissue.
B. Potentiates the actions of glucagon and epinephrine that manifest as hyperglycemia.
C. Acts on liver enzymes by decreasing glycogenesis, while increasing gluconeogenesis.
D. Increases transforming growth factor beta and insulin-like growth factor I in the wound.
E. Facilitates the breakdown of protein and amino acids, and mediates the release of lactate in skeletal muscle.

Cortisol and Glucocorticoids (cont.)

Adrenal insufficiency

- Inadequate cortisol & aldosterone
- Classically, Pt with atrophic adrenal glands caused by exogenous steroid undergo stressor e.g. surgery
- Clinical: Tachycardia, hypotension, weakness, N&V, fever
- Lab: hypoglycemia, hyponatremia, hyperkalemia
- Diagnostic test: ↓ level of baseline cortisol & ↓ ACTH – stimulated cortisol
- Rx: steroid supplementation

Glucocorticoids

- Immunosuppressive properties e.g. organ transplantation
- Immunologic changes
  - Thymic involution
  - ↓ cell-mediated immune response
  - ↓ T → lymphocyte blastogenesis
  - ↓ Mixed → lymphocyte responsiveness
  - ↓ Graft – versus – host reactions
  - Delayed hypersensitivity response

Glucocorticoids (cont.)

- Immunologic changes (cont.)
  - Inhibit leukocyte migration to site of inflammation
  - Inhibit intracellular killing in monocytes
  - Inhibit neutrophil superoxide reactivity
  - Suppress chemotaxis
- Clinical: hypoperfusion - septic shock, trauma, coronary bypass grafting

Macrophage Migration – Inhibitory Factor

Cholinergic Anti-inflammatory Pathways

- Produced by anterior pituitary & macrophages
- Reverses anti-inflammatory effects of cortisol
- Modulate inflammatory response during stress by inhibiting the immunosuppressive effect of cortisol on immunocytes
  → ↑ activity against foreign pathogens
### Growth Hormones and Insulin – Like Growth Factors

**Growth hormone**
- GH expressed by pituitary gland
- GH promotes protein synthesis and insulin resistant. Enhances the mobilization of fat store
- GH secretion is upregulated by hypothalamic GH releasing hormone and downregulated by somatostatin
- Downstream effect – direct interaction with GH receptor and secondarily through the hepatic synthesis of IGF–1

**Insulin – Like Growth Factors**
- IGF circulates primarily bound to IGF – binding proteins
- IGF anabolic effects: ↑ protein synthesis & lipogenesis
- In liver IGH stimulates protein synthesis & glycogenesis
- In adipose tissue ↑ glucose uptake & lipid utilization
- In skeletal muscle mediates glucose uptake & protein synthesis

### Critical illness
- → acquired GH resistance → ↓ level of IGH → catabolic phenotype during critical illness
- GH → liososomal superoxide production → enhances phagocytic activity
- GH also ↑ proliferation of T – cell population
- Exogenous GH → worse outcome: ↑ mortality, prolonged mediator dependence, ↑ susceptibility to infection

### Catecholamines
- Secreted by chromaffin cells of adrenal medulla
- Function as neurotransmitters in CNS
- Most common: epinephrine, norepinephrine & dopamine
- Severe injury, catecholamines level ↑ 3 – 4 fold and lasting 24 – 48 hrs
- Epinephrine → catabolic state & hyperglycemia
  - hepatic glycogenolysis, gluconeogenesis, peripheral lipolysis & proteolysis
  - ↑ insulin resistance in skeletal muscle
  - ↑ secretion of thyroid, parathyroid hormone & renin
  - Inhibit release of aldosterone

#### Epinephrine
- Activation of beta 2 receptors on immunocytes
- Inhibit release of inflammatory cytokines (TNF, IL-1, IL-6), also enhance the release of the anti-inflammatory mediator IL-10

#### Catecholamines (cont.)
- Hemodynamic effects: ↑ cardiac O2 demand, vasoconstriction, ↑ cardiac output
- Used to treat hypotension during septic shock because of ↑ cardiac stress
- Cardioprotective strategies - beta bocker for Pt undergoing surgery → significant benefit in ↓ cardiac –related deaths
Aldosterone

• Released by the zona glomerulosa of adrenal cortex
• ↑ Intravascular volume
  – Acts on the renal mineralocorticoid receptor of early distal convoluted tubules
  – Retain sodium & eliminates K+ and H+ ions
• Stimulated by ACTH, angiotensin II, ↓ intravascular volume & hyperK
• Deficiency → hypotension, hyperkalemia
• Excess → edema, HT, hypokalemia, metabolic alkalosis

Insulin

• Critical illness → hyperglycemia & insulin resistance due to catabolic effects of mediators (catecholamines, cortisol, glucagon & GH)
• Mediates anabolic state through hepatic glycogenesis & glycolysis, peripheral glucose uptake, lipogenesis & protein synthesis
• Hyperglycemia → immunosuppressive effects: glycosylation of IgG, ↓ phagocytosis & respiratory burst of monocytes and ↑ risk of infection
• Insulin therapy of hyperglycemia ↓ mortality & infection, however avoid hypoglycemia

Acute Phase Proteins

• Class of proteins produced by liver
• Either ↑ or ↓ in response to injury, infection
• C-reactive protein specific marker of proinflammatory response
  – No diurnal variation, not modulated by feeding
• May be unreliable in hepatic insufficiency

Mediator of inflammation

Cytokines

• Broad sequence of cellular responses: cell migration, DNA replication, cell turnover, immunocyte proliferation
• Local- eradicate microorganisms, promote wound healing
• Exaggerated response- hemodynamic instability (septic shock) or metabolic derangements (muscle wasting)
• Anti-inflammatory cytokines release may result in immunocyte dysfunction & host immunosuppression

TABLE 1-3: Cytokines and Their Sources

| Source | | | | |
|---|---|---|---|
| THF-α | Microphages/monocytes | NF cells | Activates TLR-4, activity on innate immune system |
| IL-1 | Microphages/monocytes | NF cells | Two forms (IL-1α and IL-1β): similar physiologic effects to THF-α, induces fever through proinflammatory activity in antoimmune response, promotes acute phase response,↑ inflammatory response |
| IL-2 | T lymphocytes | Macrophages | Promotes lymphocyte proliferation, immunoglobulin production, gut barrier integrity, half-life <10 min, attenuates production following major blood loss leading to immunosuppression, regulates lymphocyte apoptosis |
| IL-3 | T lymphocytes | Macrophages | Promotes lymphocyte proliferation |
Heat Shock Proteins

- Intracellular proteins expressed during times of stress
- Participate in protein folding & targeting
- Formation requires gene induction by a transcription factor
- Function as intracellular chaperones for ligands bacterial DNA & endotoxin
- Protect cells from deleterious effects of traumatic stress
- When released by damaged cells alert immune system of tissue damage

Reactive Oxygen Metabolites

- Highly reactive small molecules – unpaired outer orbit electrons
- Cause injury to both host cells & pathogen by oxidation of unsaturated fatty acids within cell membranes
- By-product of oxygen metabolism & anaerobic process
- Potent O2 radicals – oxygen, superoxide, hydrogen peroxide & hydroxyl radicals
- Endogenous antioxidants – superoxide dismutase, catalase & glutathione peroxidase
- During stress or ischemia, enzymatic clearance mechanism are consumed
- Restoration of perfusion, unbalanced production of ROS → reperfusion injury

Eicosanoids

- Oxidation of membrane phospholipid arachidonic acid
- Subgroups: prostaglandins, prostacyclins, hydroxyeicosatetraenoic acids (HETEs), thromboxanes, leukotrienes
- Synthesis requires activation of phospholipase A2
- Not stored within cells
- Rapid generation stimulated by hypoxic injury, direct tissue injury, endotoxin, norepinephrine, vasopressin, ang II, bradykinin, serotonin, ACh, cytokines, histamine

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**TABLE 1-3 Cytokines and Their Sources (cont.)**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Cells</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>Macrophages, B lymphocytes, monocytes, mast cells, fibroblasts, endothelial cells, osteoblasts, megakaryocytes, platelets, keratinocytes</td>
<td>Elicited by virtually all immunogenic cells; long half-life, circulating levels proportional to injury severity; predicts activated neutrophil survival</td>
</tr>
<tr>
<td>IL-8</td>
<td>Macrophages, monocytes, T lymphocytes, mast cells, epithelial cells, platelets</td>
<td>Chemokactor for neutrophils, eosinophils, lymphocytes</td>
</tr>
<tr>
<td>IL-10</td>
<td>T lymphocytes, B lymphocytes, macrophages, mast cells, mast cells, keratinocytes</td>
<td>Prominent anti-inflammatory cytokine; reduces mortality in animal sepsis and ARDS models</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>T lymphocytes, NK cells, macrophages</td>
<td>Mediates IL-12 and IL-18 function; half-life, days; found in wounds 5-7 days after injury; promotes ARDS</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>T lymphocytes, fibroblasts, endothelial cells, stromal cells</td>
<td>Promotes wound healing and inflammation through upregulation of leukocytes</td>
</tr>
<tr>
<td>IL-21</td>
<td>T lymphocytes</td>
<td>Preferentially secreted by Th2 cells; structurally similar to IL-2 and IL-15; activates NK cells, B and T lymphocytes; influences adaptive immunity</td>
</tr>
<tr>
<td>HMGB-1</td>
<td>Monocytes/macrophages</td>
<td>High-mobility group box chromosomal protein; DNA transcription factor; late (downstream) mediator of inflammation (ARDS, gut barrier disruption), induces “stiffness behavior”</td>
</tr>
</tbody>
</table>

**TABLE 1-3 Cytokines and Their Sources (cont.)**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Cells</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-12</td>
<td>Macrophages, monocytes, neutrophils, keratinocytes, dendritic cells, B lymphocytes</td>
<td>Promotes Th1 differentiation; synergistic activity with IL-2</td>
</tr>
<tr>
<td>IL-13</td>
<td>T lymphocytes</td>
<td>Promotes B-lymphocyte function; structurally similar to IL-4, inhibits nitric oxide and endothelial activation</td>
</tr>
<tr>
<td>IL-15</td>
<td>Macrophages, monocytes, epithelial cells, keratinocytes, monocytes, keratinocytes, adrenal cortical cells, osteoblasts</td>
<td>Anti-inflammatory effect; promotes lymphocyte activation; promotes neutrophil phagocytosis in fungal infections</td>
</tr>
<tr>
<td>IL-18</td>
<td>Macrophages, Kupffer cells, keratinocytes, dendritic cells, osteoblasts</td>
<td>Similar to IL-12 in function; elevated in sepsis, particularly gram-positive infections; high levels found in cardiac deaths</td>
</tr>
</tbody>
</table>
**Eicosanoids (cont.)**

- Phospholipids
  - Glucocorticoids (Cortisol)
  - Arachadonic Acid
    - Cyclic endoperoxides (PGG2, PGE2)
    - Hydroperoxyeicosatetraenoic acid
    - Prostaglandins (PGD2, PGE2, PGF2)
    - Hydroxyeicosatetraenoic Acid (HETE)
    - Thromboxane (TXA2)
    - Leukotrienes

**Systemic actions of Eicosanoids**

- Pancreas → glucose-stimulated insulin secretion, inhibit by PGE2
- Pulmonary bronchoconstriction: PGF2α, TXA2, LTC4, LTD4, LTE4
- Liver → glucagon secretion, PGE2
- Adipose → lipolysis, PGE2
- Bone → resorption, PGE2, PGE1, PGI2
- Pituitary
  - Prolactin: PGE1
  - LH: PGE1, PGE2, 5-HETE
  - TSH: PGI2, PGI1, PGE1
  - GH: PGE1
- Parathyroid → PTH secretion, PGE2
- Eicosanoids activation → formation of anti-inflammatory compound lipoxin, inhibit chemotaxis & nuclear factor kβ activation
- Glucocorticoids, NSAIDs, leukotrienes inhibitors block end products of the pathway
- Broad range of physiologic roles: neurotransmission, vasomotor regulation, immune cell regulation
- Proinflammatory response → acute lung injury, pancreatitis & ARF

**Fatty Acid Metabolites**

- Eicosanoids are produced by 2 pathways
  1. with arachidonic acid (omega-6 FA) & eicosapentaenoic acid (omega-3 FA) as substrate
  2. Omega-6 substrate
    - Downstream mediator production
    - Soy-based lipid preparations
- Omega-3 FA specific inflammatory effects
  - Inhibit NF-kβ, TNF release, leukocyte adhesion & migration
  - Chronic autoimmune disease: RA, psoriasis, lupus
  - IL-1β production of TNF, IL-1β and IL-6 by endotoxin-stimulated monocytes
- Pre-op supplementation with omega-3 FA; need for mechanical ventilation, hospital length of stay and ↓ mortality

**Kallikrein-Kinin System**

- Contribute to inflammation, BP control, coagulation & pain responses
- Prekallikrein is activated to produce serine protease kallikrein → coagulation cascade
- Kininogen is produced by liver, metabolized by kallikrein to form bradykinins
- Kinins mediate vasodilation, ↑ capillary permeability, tissue edema, pain, inhibit gluconeogenesis
  - Bronchoconstriction, ↑ renal vasodilation → ↑ renal perfusion → activate RAAS → Na resorption → ↑ intravascular volume
Kallikrein-Kinin System (cont.)
• Bradykinins & kallikrein ↑ levels during gram negative bacteremia, hypotension, hemorrhage, endotoxemia & tissue injury
  – Degree of elevation of levels - proportional to severity of injury
• In clinical trials, bradykinin antagonists help reverse G – sepsis, but do not improve survival

Serotonin
• Monoamine neurotransmitter (5-hydroxy tryptamine) derived from tryptophan
• Synthesized by neurons in CNS, enterochromaffin cells of GI tract & platelets
• Stimulates vasoconstriction, bronchoconstriction, platelet aggregation
  • ↑Myocardial ionotropy and chronotropy through nonadrenergic cAMP pathways
• Receptors located in CNS, GI tract, monocytes
• Released at sites of injury by platelets
• Unclear role in inflammation

Histamine
• Synthesized by decarboxylation of amino acid Histidine
• Released & stored in neurons, skin, gastric mucosa, mast cells, basophils, and platelets
• 4 receptor subtypes
  • H1 → vasodilation, bronchoconstriction, ↑intestinal motility and myocardial contractility
  • H2 → gastric parietal cell acid secretion
• H3 autoreceptor on presynaptic histamine-containing nerve endings → down-regulation of histamine release
• H4 expressed in bone marrow, eosinophil & mast cells
  • ↑ histamine release ← hemorrhagic shock, trauma, thermal injury, endotoxia & sepsis

Quiz
• Regarding Cytokines, which is NOT CORRECT ?
  A. TNF induces significant shock and catabolism
  B. IL-1 promotes beta-endorphin release from pituitary
  C. IL-2 promotes lymphocyte proliferation, immunoglobulin production, gut barrier integrity
  D. IL-6 circulating levels proportional to injury severity
  E. IL-12 promotes T helper 1 differentiation; synergistic activity with IL-1

Tumor Necrosis Factor
• Synthesized by macrophages, monocytes, Tcells
• Abundant in peritonium & splanchnic tissues
• Brief half-life, but many metabolic & immunomodulatory activities
  • → muscle breakdown & cachexia by ↑catabolism, insulin resistance, redistribution of amino acids to hepatic circulations
• Coagulation activation, cell migration, macrophage phagocytosis
Tumor Necrosis Factor (cont.)
• Enhances expression of adhesion molecules, PGE2, Platelet-activating factor, glucocorticoids, eicosanoids
• TNF receptors – composed of 2 subtypes
  – TNF-1 mediates proteolytic caspases → apoptosis in tissue
  – TNF-2 → NF-κB activation → inflammatory signals amplification
• TNFRs proteolytically cleaved from cell membranes → soluble form → Affinity for TNF → limit transmembrane TNFR

Interleukin-1
• IL1α- membrane associated, function through cellular contact
• IL1β- soluble form, inflammatory sequence similar to TNF
• synthesized by monocytes, macrophages, endothelial cells, fibroblasts, and epidermal cells
• Response to cytokines (TNF, IL-2, IFN-γ) and foreign pathogens

Interleukin-1 (cont.)
• Require formation of inflammasome in cell
• Synergistic effect with TNF in hemodynamic compromise
• Induces febrile response by stimulating PG activity in the anterior hypothalamus
• Regulation by IL-1 R antagonist & IL-1R2 (proteolytically cleaved from membrane to soluble form)

Interleukin-2
• Upregulated in response to IL-1
• Promotes T-lymphocyte proliferation & differentiation, Ig production, gut barrier integrity
• Bind to IL-2 R → expressed on leukocytes
• T ½ < 10 min → not detectable
• IL-2 R blockade induce immunosuppression → used for organ transplantation
• Major injury or blood transfusions reduce IL-2 activity leading to a transient immunocompromised state

Interleukin-4
• Released by activated Helper T lymphocytes
• Stimulates T-cell proliferation & B-cell activation
• Important in antibody-mediated immunity and antigen presentation
• Induces class switching B cells to produce IgG4 & IgE
  → Important in allergic and antihelmintic responses
• Anti-inflammatory effects on macrophages → by response to proinflammatory mediators (IL-1, TNF, IL-6, IL-8)
  ▲ macrophage susceptibility to anti-inflammatory effects of glucocorticoids

Interleukin-6
• Release by macrophages, stimulated by endotoxin, TNF, IL-1
• Levels are detectable within 60 min of injury, peak 4-6 hours, and persist up to 10 days
• Levels are proportional to degree of injury
• Pro-inflammatory
  → Mediates hepatic acute phase response during injury and convalescence
  → Induces and prolongs neutrophil activity
• Anti-inflammatory
  → Inhibit TNF-α and IL-1 activity
  → Promote release of soluble TNF receptors & IL-1 R antagonists
  → Stimulates release of cortisol
**Interleukin-8**

- Synthesized by macrophages, endothelial cells
- Stimulates release of IFN-γ
- Chemoattractant for neutrophils
- Proposed biomarker for risk of multiple organ failure

**Quiz**

Which of the following cytokines can reduce mortality in animal sepsis model?

A. IL-1
B. IL-6
C. IL-10
D. IL-12
E. IL-18

**Interleukin-10**

- Anti-inflammatory cytokines
- Synthesized by monocytes, also released by other lymphocytes
- Specifically enhanced by TNF & IL-1
- Inhibit secretion of TNF & IL-1, partly through downregulation of NF-κB
- Negative feedback regulator of inflammatory cascade
- ↑ plasma levels associated with mortality and disease severity

**Interleukin-12**

- Regulator of cell mediated immunity
- Released by activated phagocytes
- ↑ expressed during endotoxemia & sepsis
- Stimulate lymphocytes, ↑ IFN costimulus of IL-18 and ↑ NK cell cytotoxicity & helper T cell differentiation
- Inhibited by IL-10
- IL-12 deficiency inhibits phagocytosis in neutrophils
- In studies, IL-12 may contribute to antibacterial response
- Most evidence suggests IL-12 contributes to overall proinflammatory response

**Interleukin-13**

- Exerts many of the same immunomodulatory effects as IL-4
- Inhibits monocyte release of TNF, IL-1, IL-6, and IL-8
- ↑ secretion of IL-1 R antagonist
- Unlike IL-4, no identifiable effect on T lymphocytes & only has influence on selected B lymphocytes
- ↑ expression during septic shock—neutropenia, monocytopenia, leukopenia
- Inhibit leukocyte interaction with activated endothelial surfaces
- Net anti-inflammatory effect, similar to IL-4, IL-10

**Interleukin-15**

- Synthesized in many cell types (e.g., macrophages & skeletal muscle after endotoxin administration)
- Stimulates NK cell activation, B & T-cell proliferation
- Function as regulator of cellular immunity
- Same immunomodulatory effects as IL-2, due to shared receptor subunits
- Potent inhibitor of lymphocyte apoptosis by enhancing the expression of antiapoptotic molecules (Bcl-2)
Interleukin-18

• Formerly IFN-γ-inducing factor
• Synthesized by macrophages
• IL-18 & receptor complex are members of IL-1 superfamily
• Macrophages release IL-18 in response to endotoxin, TNF, IL-1, IL-6 and sepsis
• Activates NF-κB through myeloid differentiation primary response gene (88) (MyD88) – dependent pathway

Interleukin-18 (cont.)

• Regulation is in part mediated through IL-18 – binding protein (IL-18BP)
• Mediates hepatotoxicity associated with Fas ligand and TNF
• Molluscum contagiosum secretes IL-18BP – like protein → neutralize IL-18 → inhibit inflammatory response
• IL-18 & IL-12 synergistically release IFN-γ from T cells

Interferons

• First recognized as soluble mediators, inhibit viral replication through activation of specific antiviral genes
• Catigorized into 2 nature subtypes based on receptor specificity & sequence homology
  – Type I (IFN-α, IFN-β, IFN-ω) structurally related and bind to a common receptor, IFN- α receptor, response to viral antigens, dsDNA, bacteria, tumor cells LPS
  – Type II (IFN-γ) secrete by T lymphocytes, NK cells, APC in response to bacterial antigen IL-2, IL-2 IL-18

Interferons (cont.)

• Type I interferons induce maturation of dendritic cells
• Stimulate class I MHC expression
• IFN-α & IFN-β ↑ cytotoxicity of NK cells
• Type I interferons have been studied as therapeutic agents in hepatitis C and relapsing multiple sclerosis
• Type II interferon stimulate release of IL-12, IL-18
• Negative regulators of IFN- γ include IL-4, IL-10, glucocorticoids
• IFN- γ binding with cognate receptor activates the Janus kinase / signal transducer & activator of transcription (JAK/STAT) pathway → induction of biologic response

Interferons (cont.)

• Macrophage stimulated by IFN- γ → enhanced phagocytosis & microbial killing, ↑ release of oxygen radicals through NADP-dependent phagocyte oxidase
• Mediates macrophage stimulation → acute lung injury after major surgery or trauma
• Regulate trafficking of immunocytes to sites of inflammation via upregulation of chemotactantants
• Promotes differentiation of T cells to helper T cell subtype 1 and B-cell isotype switching to IgG

Quiz

• Circulating levels of which cytokines are commonly elevated in patients with sepsis?
  A. Interleukin-1
  B. Interleukin-6
  C. Interleukin-12
  D. Interleukin-18
  E. Interferon-gamma
Granulocyte-Macrophage Colony-Stimulating Factor

• Upregulates both granulocyte and monocyte cell lines from hematopoietic bone marrow stem cells
• Response to TNF
• Inhibit both monocyte and neutrophil apoptosis
• Enhance macrophage cytokine release in response to stimuli
• Potentiate release of neutrophil superoxide and cytotoxicity of monocyte
• GM-CHF has proven beneficial during the treatment of fungal infections in immunocompromised patients

Granulocyte-Macrophage Colony-Stimulating Factor (cont.)

• May potentiate acute lung injury during critical illness
• Effective in promoting the maturation and recruitment of functional leukocyte for normal cytokine response
• Maybe effective in wound healing

High Mobility Group Box 1

• DNA transcription factor, facilitate the binding of regulatory protein complexes to DNA
• Actively secreted by macrophages, NK cells, and enterocytes
• Endotoxin, TNF, IFN-γ promote the release of HMGB1
• Promote release of TNF from monocytes → cytokine-like activity

High Mobility Group Box 1 (cont.)

• Passively released by necrotic cell
• Contribute to regulation of inflammation after tissue injury
• Receptor for HMGB1 are receptors for advanced glycation end produces & Toll – like receptor 4
• Binding lead to the pro inflammatory response through activation of NF-κ B
Cell Signaling Pathways
- G-Protein Receptors
- Ligand-Gated Ion Channels
- Receptor Tyrosine Kinases
- Janus Kinase/Signal Transducer and Activator of Transcription Signaling
- Suppressors of Cytokine Signaling
- Mitogen-Activated Protein Kinases
- Nuclear Factor B
- Toll-Like Receptors and CD14
- Apoptosis

Cell-Mediated Inflammatory Response
- Cell-Mediated Inflammatory Response
- Platelets
- Lymphocytes and T-Cell Immunity
- Eosinophils
- Mast Cells
- Monocytes
- Neutrophils

Endothelium-Mediated Injury
- Vascular Endothelium
- Neutrophil-Endothelium Interaction
- Nitric Oxide
- Prostacyclin
- Endothelins
- Platelet-Activating Factor
- Atrial Natriuretic Peptides

Cellular Response to Injury
- These mRNA transcripts are also regulated by modulation mechanisms, including
  - (a) splicing, which can cleave mRNA and remove noncoding regions;
  - (b) capping, which modifies the 5' ends of the mRNA sequence to inhibit breakdown by exonucleases;
  - (c) the addition of a polyadenylated tail
- highly specific DNA sequences upstream of the target gene known as the promoter region
Gene Expression and Regulation

- Gene expression and protein synthesis can occur within a 24-hour period. The process can be regulated at various stages: transcription, messenger RNA (mRNA) processing, or protein packaging. At each stage, it is possible to inactivate the mRNA or protein, rendering these molecules nonfunctional.

Gene Expression and Regulation

- During systemic inflammation, transcription factors are highly important, because regulation of cytokine gene expression may have profound effects on the clinical phenotype.

Cell Signaling Pathways

- G-Protein Receptors
- Ligand-Gated Ion Channels
- Receptor Tyrosine Kinases
- Janus Kinase/Signal Transducer and Activator of Transcription Signaling
- Suppressors of Cytokine Signaling
- Mitogen-Activated Protein Kinases
- Nuclear Factor B
- Toll-Like Receptors and CD14
- Apoptosis

G-Protein Receptors (GPR)

- Vasoactive polypeptide, epinephrine, bradykinin, leukotriene, PG, PL
- Inflammatory response $\rightarrow$ Extracellular ligands bind to GPR $\rightarrow$ conformational change and activation of associated proteins $\rightarrow$ ER release
- 2nd messenger (1) cAMP, and (2) calcium, released from the endoplasmic reticulum

G-Protein Receptors (GPR)

- cAMP $\uparrow$ activate gene transcription by protein kinaseA
- Calcium $\uparrow$ activate phospholipase C with further subsequent downstream effects.
- GPR binding also can promote the activity of protein kinase C, which can subsequently stimulate NF- B as well as other transcription factors.
**G-Protein Receptors (GPR)**

- Allow the rapid influx of ions (Na, Cl, Ca, K)
- Neurotransmitters (nicotinic Ach receptor)
- Chemical signal into an electrical signal.

**Ligand-Gated Ion Channels**

- Several growth factors, including platelet-derived growth factor, insulin-like growth factor, epidermal growth factor, and vascular endothelial growth factor.
- Dimerize with adjacent receptors → autophosphorylation → recruit secondary signaling molecules (e.g., phospholipase C).
- Activation of RTK is important for gene transcription as well as cell proliferation and may have influence in the development of many types of cancer.

**Receptor Tyrosine Kinases**

- A family of tyrosine kinases
- Cell proliferation, inflammations
- Bind signal transduction (IFN-γ, IL-6, IL-10, IL-12, and IL-13), cell stressor
- JAKs + cytokines + ligand dimerization, activated JAKs recruit and phosphorylate STAT molecules
- Activated STAT proteins further dimerize and translocate into the nucleus and modulate the transcription of target genes.

The Janus kinases (JAKs) signal transducer and Activator of Transcription Signaling (STAT)
**JAK/STAT**

- STAT-DNA binding can be observed within minutes of cytokine binding.
- The JAK/STAT system is a rapid pathway for membrane to nucleus signal transduction.
- The JAK/STAT pathway is inhibited by the action of phosphatase, the export of STATs from the nucleus, as well the interaction of antagonistic proteins.
- Intracellular molecules that inhibit STAT function, known as *suppressors of cytokine signaling* (SOCSs).

**Suppressors of Cytokine Signaling**

- A group of cytokine-induced proteins
- Negative feedback loop by downregulating the JAK/STAT pathway.
- Inhibitory effect partly by binding with JAK and thus competing with STAT.
- A deficiency of SOCS activity results in cell hypersensitivity to certain stimuli (inflammatory cytokines and growth hormones).

**Mitogen-Activated Protein Kinases**

- Inflammatory signaling and regulation of cell proliferation and cell death.
- MAPK pathways involve sequential stages of mediator phosphorylation resulting in the activation of downstream effectors, including JNK, ERK, and p38 kinase, with subsequent gene modulation.
- Dephosphorylation of MAPK pathway mediators inhibit their function.

**MAPK**

- Activated JNK phosphorylates c-Jun, which dimerizes to form the transcription factor activated protein 1.
- The protein MAP/ERK kinase kinase (MEKK) has several functions, including protein kinase and ubiquitin ligase, and also has been shown to downregulate MAPK pathways.
- JNK is activated by TNF and IL-1 and is a regulator of apoptosis.
MAPK

- Pharmacologic blockade of JNK was associated with decreased pulmonary injury and TNF and IL-1 secretion in an ischemia/reperfusion model.
- Endotoxin, viruses, IL-1, IL-2, IL-7, IL-17, IL-18, and TNF activated the p38 kinase.
- p38 favors immunocyte development
- p38 inactivation is a critical step in the differentiation of thymic T cells.
- These MAPK isoforms do not function independently but rather exhibit significant counteraction and cosignaling, which can influence the inflammatory response.

MAP

Nuclear Factor κB

- a transcription factor key role in regulating the gene products expressed after inflammatory stimuli
- p50 and p65 in the cytosol in the resting state primarily through the inhibitory binding of inhibitor of κB (I-κB).
- response to an inflammatory stimulus (TNF, IL-1, endotoxin)
- a sequence of intracellular mediator phosphorylation reactions leads to the degradation of I-κB and subsequent release of NF-κB.

Nuclear Factor κB

- NF-κB travels to the nucleus and promotes gene expression and gene expression for I-κB (negative feedback)
- In clinical appendicitis, for example, increased NF-κB activity was associated with initial disease severity, and levels returned to baseline within 18 hours after appendectomy in concert with resolution of the inflammatory response.

NF κB

Toll-Like Receptors and CD14

- The innate immune system responds to pathogen-associated molecular patterns (PAMPs) such as microbial antigens and LPS.
- Toll-like receptors (TLRs) are a group of pattern recognition receptors activated by PAMPs
- Function as effectors of the innate immune system and belong to the IL-1 superfamily.
Toll-Like Receptors and CD14

• Immunocyte recognition of LPS is mediated primarily by TLR4.
• LPS-binding proteins chaperone LPS to the CD14/TLR4 complex to activate MAPK, NF-κB, and cytokine gene expression.
• In contrast to TLR4, TLR2 recognizes PAMPs from gram-positive bacteria, including lipoproteins, lipopeptides, peptidoglycans, and phenol-soluble modulin from *Staphylococcus* species.

• Interestingly, loss-of-function single nucleotide polymorphisms of TLR4 increased risk of infection in susceptible critically ill patients.
• TLRs also bind DAMPs (endogenous cellular products released during times of stress or injury).
• DAMPs include products such as HMGB1, heat shock proteins, and hyaluronic acid.
• Innate immune activation by DAMPs stimulates the recruitment of inflammatory cells to the site of injury and also mediates proinflammatory signaling.

Apoptosis

• Apoptosis (regulated cell death)
  – energy-dependent, organized mechanism for clearing senescent or dysfunctional cells, including macrophages, neutrophils, and lymphocytes, without promoting an inflammatory response.
• Cell necrosis
  – a disorganized sequence of intracellular molecular releases with subsequent immune activation and inflammatory response.

• The extrinsic pathway
  – activated through the binding of death receptors (e.g., Fas, TNFR), recruitment of Fas-associated death domain protein and subsequent activation of caspase 3
  – caspases are the effectors of apoptotic signaling (mediate the organized breakdown of nuclear DNA)

• The intrinsic pathway
  – proceeds through protein mediators (e.g., Bcl-2, Bcl-2-associated death promoter, Bcl-2–associated X protein, Bim) → increased mitochondrial membrane permeability mitochondrial release of mitochondrial cytochrome C (ultimately activates caspase 3).

These pathways do not function in a completely autonomous manner, because there is significant interaction and crosstalk between mediators of both extrinsic and intrinsic pathways.
Apoptosis

- Regulatory factors of apoptosis
  - inhibitor of apoptosis proteins and regulatory caspases (e.g., caspases 1, 8, 10).
- Apoptosis during sepsis may influence the ultimate competency of the acquired immune response
  - In a murine model of peritoneal sepsis, increased lymphocyte apoptosis was associated with mortality, which may be due to a resultant decrease in IFN-\(\gamma\) release
- Pt who sepsis dead
  - lymphosis cell apoptosis relate sepsis dead but not macrophage

Apoptosis

- Clinical trials have observed an association between the degree of lymphopenia and disease severity in sepsis
- the phagocytosis of apoptotic cells by macrophages, anti-inflammatory mediators such as IL-10 are released that may exacerbate immune suppression during sepsis.
- Neutrophil apoptosis is inhibited by inflammatory products, including TNF, IL-1, IL-3, IL-6, GM-CSF, and IFN-\(\gamma\). Or from free radical

Cell-Mediated Inflammatory Response

- Platelets
- Lymphocytes and T-Cell Immunity
- Eosinophils
- Mast Cells
- Monocytes
- Neutrophils

Platelets

- nonnucleated
- mitochondria and mediators of coagulation and inflammatory signaling.
- derived from bone marrow megakaryocytes.
- Role as Hemostatic response and are activated by several factors, including exposed collagen. Activated platelets at the site of injury release inflammatory mediators

Platelets

- Principal chemoattractant for neutrophils and monocytes.
  - The migration to site of injury
  - enhanced by serotonin release, platelet-activating factor, and prostaglandin E2.
  - Plts are source of eicosanoids and vasoactive mediators.
- A hallmark of the septic response includes thrombocytopenia (multifactor).
- NSAIDs inhibit platelet function through the blockade of COX.
Lymphocytes and T-Cell Immunity

- primarily of B cells, T cells, and natural killer cells
- TH1 cells favor cellular immune responses and secrete IFN-γ, IL-2, and IL-12 (defense against bacterial pathogens)
- TH2 cells favor humoral responses and produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 (critical illness induced by severe trauma or sepsis)

- In burn injury, T-cell suppression via the release of transforming growth factor beta (TGF-β) downregulate Tfn
- Arginine is essential for T-cell proliferation and receptor function.

Eosinophils

- antihelmintic.
- mostly in tissues such as the lung and GI tract.
- role in immune surveillance.
- eosinophils can be activated by IL-3, IL-5, GM-CSF, chemoattractants, and platelet-activating factor.
- Eosinophil activation can lead to subsequent release of toxic mediators, including reactive oxygen species, histamine, and peroxidase

Mast Cells

- primary response cause located in tissues.
- release TNF → neutrophil recruitment and pathogen clearance.
- role in the anaphylactic response to allergens.
- stimulant (allergen binding, infection, and trauma)
- produce (histamine, cytokines, eicosanoids, proteases, and chemokines), which leads to vasodilatation, capillary leakage, and immunocyte recruitment.
- Mast cells are thought to be important cosignaling effector cells of the immune system via the release of IL-3, IL-4, IL-5, IL-6, IL-10, IL-13, and IL-14, as well as macrophage migration–inhibiting factor.

Monocytes

- differentiate into macrophages, osteoclasts, and dendritic cell
- primarily through mechanisms that include phagocytosis of microbial pathogens, release of inflammatory mediators, and clearance of apoptotic cells.
- In humans, downregulation of monocyte and neutrophil TNFR expression has been demonstrated experimentally and clinically during systemic inflammation.
Monocytes

- In clinical sepsis, nonsurviving patients with severe sepsis have an immediate reduction in monocyte surface TNFR expression with failure to recover.
- Whereas surviving patients have normal or near-normal receptor levels from the onset of clinically defined sepsis.
- Congestive heart failure reduces in monocyte surface TNFR expression.

Monocytes

- In experimental models, endotoxin has been shown to differentially regulate over 1000 genes in murine macrophages with approximately 25% of these corresponding to cytokines and chemokines.
- During sepsis, macrophages undergo phenotypic reprogramming highlighted by decreased surface human leukocyte antigen DR (a critical receptor in antigen presentation), which also may contribute to host immunocompromise during sepsis.

Neutrophils

- Potent mediators of acute inflammation.
- Chemotactic mediators from a site of injury induce neutrophil adherence to the vascular endothelium and promote eventual cell migration into the injured tissue.
- Neutrophils are circulating immunocytes with short half-lives (4 to 10 hours).
- On activation by inflammatory stimuli, including TNF, IL-1, and microbial pathogens, neutrophils are able to phagocytose, release lytic enzymes, and generate large amounts of toxic reactive oxygen species.

Endothelium-Mediated Injury

- Vascular Endothelium:
- Neutrophil-Endothelium Interaction:
- Nitric Oxide
- Prostacyclin
- Endothelins
- Platelet-Activating Factor
- Atrial Natriuretic Peptides

Vascular Endothelium

- Physiologic conditions, anticoagulant properties
- Critical function as barriers that regulate tissue migration of circulating cells.
- During sepsis, endothelial cells are differentially modulated, which results in an overall procoagulant shift via decreased production of anticoagulant factors, which may lead to microthrombosis and organ injury.

Neutrophil-Endothelium Interaction

- Endothelial adhesion factors referred to as selectins
ICAM-1 = intercellular adhesion molecule-1; ICAM-2 = intercellular adhesion molecule-2; Mac-1 = macrophage antigen 1; PECAM-1 = platelet-endothelial cell adhesion molecule-1; VCAM-1 = vascular cell adhesion molecule-1; VLA-4 = very late antigen-4.

- L-cell + P-endo in capture
- L-cell + L endo in fast rolling
- P in slow rolling
- E in arrest
- Intregrin+ Ig in firm adhesion

**Nitric Oxide**

- Or endothelium-derived relaxing factor
- Form L-arginine
- Increase cAMP cGMP
- reduce microthrombosis by reducing platelet adhesion and aggregation
- Increased NO is detectable in septic shock and in response to TNF, IL-1, IL-2, and hemorrhage. NO mediates hypotension observed during septic shock

**Prostacyclin**

- vasodilator and also inhibits platelet aggregation
- endothelial prostacyclin expression is impaired during systemic inflammation
- Prostacyclin therapy during sepsis → to reduce the levels of cytokines, growth factors, and adhesion molecules through a cAMP-dependent pathway
- In clinical trial: show it can increase cardiac output, splanchnic blood flow, and oxygen delivery and consumption with no significant decrease in mean arterial pressure

**Endothelins**

- potent mediators of vasoconstriction
- ET-1, synthesized primarily by endothelial cells,
- most potent endogenous vasoconstrictor and is estimated to be 10x of angiotensin II
- upregulated in response to hypotension, LPS, injury, thrombin, TGF-β, IL-1, angiotensin II, vasopressin, catecholamines, and anoxia
- half-life of plasma ET is between 4 and 7 minutes
Endothelins

- ET Receptor (referred to as $ET_A$, $ET_B$, and $ET_C$) via the G-protein receptor
- $ET_A$ increased inotropy and chronotropy
- $ET_B$ increased NO and prostacyclin production
- ETs can disrupt the normal blood flow and distribution and may compromise oxygen delivery to the tissue

Platelet-Activating Factor

- natural phospholipid
- PAF is released by neutrophils, platelets, mast cells, and monocytes, and is expressed at the outer leaflet of endothelial cells
- activate neutrophils and platelets, and increase vascular permeability

Platelet-Activating Factor

- PAF-acetylhydrolase, = endogenous inhibitor of PAF
- PAF-acetylhydrolase administration in patients with severe sepsis has yielded some reduction in multiple organ dysfunction and mortality

Atrial Natriuretic Peptides

- released primarily by atrial tissue and also synthesized by the gut, kidney, brain, adrenal glands, and endothelium.
- vasodilation, fluid and electrolyte excretion by inhibitors of aldosterone secretion and prevent reabsorption of sodium
- some trial suggest that ANP can reverse acute renal failure or early acute tubular necrosis

Table 2-8. Body Fuel Expenditures in a 70-kg Man

<table>
<thead>
<tr>
<th>Component</th>
<th>Mass (g)</th>
<th>Energy (kcal)</th>
<th>Similar}[\text{calorie} = 4.5]</th>
<th><em>R</em></th>
<th><em>R</em></th>
<th>2.2 \text{g/kg per day}</th>
<th>30.0</th>
<th>8.3</th>
<th>3.0 \text{g/kg per day}</th>
<th>3.4</th>
<th>2.2 \text{g/kg per day}</th>
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<tr>
<td>Carbohydrate</td>
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<td>2.0</td>
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</tr>
</tbody>
</table>
Surgical metabolism

Principle

- After surgical or traumatic injury
  - Reduced total body energy expenditure
  - Reduced urinary nitrogen wasting

- In recovery phase
  - Augmented metabolic rates
  - Augmented oxygen consumption and enzymatic preference to preserve vital organ function and to support repair of injured tissue

**Metabolism during short fasting**

- **Carbohydrate**
  - Hepatic glycogen stores are rapidly depleted → ↓ serum glucose concentration
  - Hepatic gluconeogenesis
    - Lactate, glycerol, and amino acids such as alanine and glutamine
    - The recycling of lactate and pyruvate as the Cori cycle
    - Provide up to 40% of plasma glucose during starvation

- **Protein**
  - Protein degraded amount 75 g/d for a 70-kg adult
  - Due to ↓ insulin and ↑ cortisol release
  - ↑ urinary nitrogen excretion from 7 - 10 g/d to 30+ g/d
  - Mainly in skeletal muscles then in solid organs

- **Lipid**
  - Provide 40% or more of caloric expenditure
  - Free fatty acids and glycerol degraded amount 75 g/d for a 70-kg adult
  - Due to ↓ insulin and ↑ glucagon and catecholamine
### Metabolism during prolonged fasting

- **Protein**
  - Proteolysis to 20 g/d
  - Urinary nitrogen excretion at 2 - 5 g/d
  - Increases renal excretion of ammonium ions → ↑ gluconeogenesis by the use of glutamine and glutamate (½ of glucose production)

- **Lipid**
  - Using ketone bodies as principal fuel source for vital organs
  - ↓ rate of glycolysis, gluconeogenesis, and proteolysis by inhibiting the enzyme pyruvate dehydrogenase.

### Lipid metabolism after injury

- **Mainly Structure of cell membranes and potentially of the immune response during systemic inflammation**
- **50 - 80% energy source**
- ↓ rate of proteolysis
- Catecholamine stimulus include ACTH, thyroid hormone, cortisol, glucagon, growth hormone, ↓ insulin levels, ↑ sympathetic stimulus

### Lipid absorption

- 1 g = 9 kcal
- Dietary and exogenous sources provide the major source of triglycerides
- Require pancreatic lipase and phospholipase to hydrolyze then esterification of the monoglycerides with fatty acyl coenzyme A (acyl-CoA)
- LCTs enter the circulation through the lymphatic system as chylomicrons.
- Shorter fatty acid chains directly enter the portal circulation and are transported to the liver by albumin carriers

### Lipolysis and Fatty Acid Oxidation

- Mediated on triglyceride lipase through a cAMP pathway
- Triglycerides → hydrolyze → free fatty acids + glycerol
- Insulin
  - ↓ Lipolysis
  - ↑ Triglyceride synthesis by
    - ↑ lipoprotein lipase activity
    - ↑ intracellular levels of glycerol-3-phosphate
- Free fatty acids enter the capillary circulation and are transported by albumin to tissues
- The use of glycerol depends on the availability of tissue glycerokinase (liver and kidneys)

### Lipid absorption

- **Hepatocytes**
  - Use free fatty acids as a fuel source during stress states
  - Synthesize phospholipids or triglycerides (VLDL) during fed states
- Systemic tissue can use chylomicrons and triglycerides as fuel by hydrolysis with lipoprotein lipase at the luminal surface of capillary endothelium
- Trauma or sepsis suppresses lipoprotein lipase activity in both adipose tissue and muscle, presumably mediated by TNF

### Lipolysis and Fatty Acid Oxidation

- Free fatty acids absorbed by cells conjugate with acyl-CoA then across the mitochondrial membrane via the carnitine shuttle
- MCTs are more efficiently oxidized than LCTs
- Rapid oxidation of MCTs makes them less prone to fat deposition
- However, exclusive use of MCTs as fuel in animal studies has been associated with higher metabolic demands and toxicity, as well as essential fatty acid deficiency.
**Lipolysis and Fatty Acid Oxidation**
- Within the mitochondria
  - Fatty acyl-CoA → beta oxidation → produces acetyl-CoA → TCA cycle → 12 ATP + CO2 + H2O
  - Excess acetyl-CoA molecules serve as precursors for ketogenesis
  - VS glucose metabolism, oxidation of fatty acids requires less oxygen and produces less carbon dioxide
  - Ratio of carbon dioxide produced to oxygen consumed is known as the respiratory quotient (RQ)
    - RQ = 0.7 imply greater fatty acid oxidation for fuel
    - RQ = 1 indicates greater carbohydrate oxidation (overfeeding)
    - RQ = 0.85 suggests the oxidation of equal amounts of fatty acids and glucose

**Ketogenesis**
- 1 Carbohydrate → 1 acetyl-CoA into the TCA cycle → 1 TCA enzyme activity
- 1 Lipolysis and 1 systemic carbohydrate availability → 1 acetyl-CoA → hepatic ketogenesis
- Hepatic ketone production > extrahepatic ketone utilization = Ketosis
- The rate of ketogenesis inversely related to the severity of injury
  - Severe injury = ketosis by 1 insulin levels and by causing rapid tissue oxidation of free fatty acids
  - Minor injury = plasma free fatty acid concentrations and ketogenesis.
  - However, in minor stress states ketogenesis does not exceed that in nonstressed starvation

**Carbohydrate Metabolism**
- Glucose and galactose are absorbed by energy-dependent active transport coupled to the sodium pump
- Fructose absorption occurs by concentration-dependent facilitated diffusion
- Glucose production occurs at the expense of protein stores
- Maintenance glucose administration (50 g/d) is to minimize muscle wasting by fat entry into the TCA cycle and ketosis
- In septic and trauma patients the exogenous glucose never fully suppress amino acid degradation for gluconeogenesis because of
  - Insulin administration → 1 protein catabolism by
    - 1 protein synthesis in skeletal muscles
    - 1 hepatocyte protein degradation
    - 1 RNA synthesis in muscle cells

**Carbohydrate Metabolism**
- Glucose is phosphorylated to form G-6-P and polymerized during glycogenesis or catabolized in glycogenolysis
- Glucose catabolism occurs by pyruvate pathway or pentose shunt
- Excess glucose from overfeeding, as reflected by RQs > 1.0, can result in
  - Glucosuria, thermogenesis and lipogenesis
  - CO2 production
  - Inferior risk and immune suppression due to hyperglycemia
- Injury and severe infections acutely induce a state of peripheral glucose intolerance due to
  - Pyruvate dehydrogenase activity → 1 pyruvate to acetyl-CoA → 1 entry into the TCA cycle
  - 1 Pyruvate and lactate → 1 gluconeogenesis
  - Plasma glucose levels α the severity of injury due to glucagon

**Carbohydrate Metabolism**
- Hepatic gluconeogenic
  - Cannot be suppressed by exogenous or excess glucose administration
  - Do not require insulin for glucose transport
- The elevated glucose concentrations also
  - Due to catecholamines and glucagon that increased hepatic gluconeogenesis and peripheral insulin resistance
  - Provide a necessary energy source for leukocytes in inflamed tissues and in sites of microbial invasion
  - Glucocorticoid infusion alone does not increase glucose levels, but it does prolong effects of catecholamines and glucagon
  - Glycogen can be mobilized by
    - Epinephrine activation of beta-adrenergic receptors, GTP-binding proteins (G-proteins)
    - Then activates cAMP and phosphorylase kinase and turn to conversion G-1-P
  - Ratio of carbon dioxide produced to oxygen consumed is
    - RQ = 0.85 suggests the oxidation of equal amounts of fatty acids and glucose
    - RQ = 0.7 imply greater fatty acid oxidation for fuel
    - RQ = 1 indicates greater carbohydrate oxidation (overfeeding)
  - Excess acetyl-CoA molecules serve as precursors for ketogenesis

**Glucose Transport and Signaling**
- Diffusion glucose transporters (GLUTs)
  - Transport of glucose down a concentration gradient
    - Type  Amino Acids  Major Expression Sites
    - GLUT 1  Glucose  Placenta, brain, kidney, colon
    - GLUT 2  Glucone  Liver, pancreatic β-cell, kidney, small intestine
    - GLUT 3  Brain  Brain, testis
    - GLUT 4  Skeletal muscle, heart muscle, white fat
    - GLUT 5  Small intestine, sperm
  - Na+/glucose secondary active transport system (SGLT)
    - Transports glucose molecules against concentration gradients by active transport.
Glucose Transport and Signaling

- GLUTs
  - GLUT1 is in blood-brain barrier but little is found in the liver and skeletal muscle
  - GLUT2 is important for rapid export of glucose resulting from gluconeogenesis
  - GLUT3 mRNA has been detected in almost every human tissue
  - GLUT4 is important in the physiology of patients with insulin-resistant diabetes
  - GLUT5 has been identified in several tissues but is primarily expressed in the jejunum as a fructose transporter

- SGLTs
  - Found in the intestinal epithelium and in the proximal renal tubules
  - Transport both sodium and glucose intracellularly
  - SGLT1 is on brush borders of small intestine enterocytes
  - SGLT1 and SGLT2 are both associated with glucose reabsorption at proximal renal tubules.

Protein and Amino Acid Metabolism

- The average protein intake 80 - 120 g/d
- 6 g of protein/1 g of nitrogen
- 1 g of protein/4 kcal of energy
- After injury the initial systemic proteolysis
  - Glucocorticoids
  - Urinary nitrogen excretion to levels in excess of 30 g/d
  - Loss in lean body mass of 1.5% per day
- Protein catabolism after injury also
  - Substrates for gluconeogenesis
  - Synthesis of acute phase protein
  - Skeletal muscles first then visceral tissues
  - Urea excretion - 1 intracellular elements such as sulfur, phosphorus, potassium, magnesium, and creatinine
Estimation of Energy Requirements

- Determine the severity of nutrient deficiencies or excess
- Predicting nutritional requirements
- Pertinent information is weight loss, chronic illnesses, or dietary habits, social habits and the use of medications
- Physical examination seeks to assess loss of muscle and adipose tissues, organ dysfunction, changes in skin, hair, or neuromuscular function, anthropometric data and biochemical determinations
- The stresses and natural history of the disease process, nutritional assessment, remains the basis for identifying patients in acute or anticipated need of nutritional support.

Nutrition in the surgical patient

Textbook conference
24 Jul 2010

- Maintained energy requirements for metabolic processes, core temperature maintenance, and tissue repair
- Failure to provide adequate nonprotein energy sources will lead to consumption of lean tissue stores
- Indirect calorimetry, serum markers and estimated from urinary nitrogen excretion, which is proportional to resting energy expenditure
- BEE : Harris-Benedict equations
  - Men : 66.47 + 13.75 (w) + 5 (H) - 6.76 (A) kcal/d
  - Women : 65.51 + 9.56 (w) + 1.85 (H) - 4.68 (A) kcal/d
- Approximately 30 kcal/kg/d

Estimation of Energy Requirements

<table>
<thead>
<tr>
<th>Condition</th>
<th>Kcal/kg/d</th>
<th>Adjustment above BEE</th>
<th>Grams of Protein/kg/d</th>
<th>Nonprotein Calories: Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal/m moderate malnutrition</td>
<td>25 – 30</td>
<td>1.1</td>
<td>1.0</td>
<td>150:1</td>
</tr>
<tr>
<td>Mild stress</td>
<td>25 – 30</td>
<td>1.2</td>
<td>1.2</td>
<td>150:1</td>
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<tr>
<td>Mod. stress</td>
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<td>1.4</td>
<td>1.5</td>
<td>120:1</td>
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<td>Severe stress</td>
<td>30 – 35</td>
<td>1.6</td>
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<td>90-120:1</td>
</tr>
<tr>
<td>Burns</td>
<td>35 – 40</td>
<td>2.0</td>
<td>2.5</td>
<td>90-100:1</td>
</tr>
</tbody>
</table>

- Increased protein intake and a lower calorie:nitrogen ratio of 80:1 to 100:1 may benefit healing in selected hypermetabolic or critically ill patients
- Approximately 0.25 - 0.35 g of nitrogen/kg daily

Vitamins and Minerals
- Most commercial vitamin preparations do not contain vitamin K, B12 or folic acid.
- Essential fatty acid supplementation especially in patients with depletion of adipose stores

Overfeeding
- Overestimation of caloric needs
- Use actual body weight with significant fluid overload and the obese or indirect calorimetry to calculate the BEE
- Contribute to clinical deterioration
  - ⌗ Oxygen consumption
  - ⌗ CO2 production
  - ⌗ Prolonged ventilatory support
  - ⌗ Fatty liver
  - ⌘ Leukocyte function
  - ⌗ Hypoglycemia
  - ⌗ Risk of infection.

Enteral Nutrition

- Enteral nutrition is preferred over parenteral nutrition
  - Lower cost
  - ⌗ Risks of the intravenous route, including vascular access complications
  - ⌗ Intestinal mucosal atrophy
  - ⌗ Infectious complications approximately 44%
  - ⌗ Acute phase protein production
  - No differences in outcome and complications after GI surgery (albumin ≥ 4 g/dL)
- Exception in closed-head injury which is no significant
  - Include permanent neurologic impairment, oropharyngeal dysfunction, short-bowel syndrome, bone marrow transplantation, major trauma and in patients who have prolonged recovery after surgery.
Enteral Nutrition

1.0 kcal/mL
Limited complex carbohydrates, MCTs and LCTs

A nonprotein calorie:nitrogen ratio of 150:1

Surgical complications
At least 100 to 150 kcal/g nitrogen, both carbohydrates and proteins must be infused simultaneously
Isotonic and nonisotonic mixtures
Aromatic amino acid levels
Critically ill patients who are hypermetabolic for >5 days and/or cannot feed via enteral tube

Provide baseline carbohydrates, protein, electrolytes, water, fat, and fat-soluble vitamins

Enteral Formulas

Carbohydrate content
Suitable for fluid restriction or unable to tolerate large-volume infusions

Fat content to 50% of the total calories
Include glutamine, arginine, branched-chain amino acids, omega-3 fatty acids, nucleotides, and beta carotene

Enteral Formulas

Indication
Use in malnutrition, sepsis, or surgical or traumatic injury in seriously ill patients who cannot feed via gastrointestinal tract or to supplement inadequate oral intake

Continuous infusion of a hyperosmolar solution
At least 100 to 150 kcal/g nitrogen, both carbohydrates and proteins must be infused simultaneously
Preoperative nutritional support may benefit in extensive malnutrition patients
Higher rates of infectious complications
Stress hormone and inflammatory mediator response to an antigenic challenge
Fewer infectious complications than no feeding at all
No benefit in cancer patients but fewer infectious complications

Parenteral Formulas

Use in malnutrition, sepsis, or surgical or traumatic injury in seriously ill patients who cannot feed via gastrointestinal tract or to supplement inadequate oral intake
Must be awareness of the associated complications
To maintain energy requirements for metabolic processes, core temperature maintenance, and tissue repair
Indication
Newborn infants with catastrophic gastrointestinal anomalies
Infants who fail to thrive due to gastrointestinal insufficiency
Short-bowel syndrome secondary to massive small-bowel resection
Enteritis, enterocolitis, enterovescical, or high-output enterocutaneous fistulas (>500 mL/d)
Protracted paralytic ileus after major operations, multiple injuries, or blunt or open abdominal trauma, or patients with reflex ileus complicating various medical diseases
Malabsorption
Functional gastrointestinal disorders
Disease at major portions of the absorptive mucosa
Malignancy with malabsorption
Steady-state calories by enteral tube feedings
Critically ill patients who are hypermetabolic for >5 days and cannot feed via enteral tube

Parenteral Formulas

Up to 1 kcal
Different approach with injury risks, not commonly done

Hepatic-Failure Formulas
Contains essential amino acids but no trace elements or vitamins
High ketogenic calorie:nitrogen ratio
Nutrient-dense Formulas
High protein-calorie-nitrogen ratio
High energy-density formulas
Carbohydrate content
Cannot produce CO2, ventilation burden
Hepatic-Failure Formulas
Aromatic amino acids and branched-chain amino acids, which can potentially reverse encephalopathy in patients with hepatic failure
No clear benefit now been proven by clinical trials

Parenteral Nutrition

Continuous infusion of a hyperosmolar solution
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Parenteral Nutrition

- **Contraindication**
  - Inevitable dying
  - Unstable hemodynamic or severe metabolic derangement
  - Can feed via gastrointestinal tract ion

- **Good nutritional status**
  - Infants with <8 cm of small bowel

- **Formulas**
  - **Total Parenteral Nutrition**
    - High dextrose content (15 to 25%)
    - Via large-diameter vein to deliver all macronutrients and micronutrients
  - **Peripheral Parenteral Nutrition**
    - Low dextrose content (5 to 10%) and protein (3%)
    - Via peripheral veins
    - Limit some nutrients
    - Considered if central routes are not available or if supplemental nutritional support is required
    - Used for short periods (<2 weeks)

**Intravenous Access Methods**

- Subclavian or internal jugular vein and threaded into the superior vena cava
- More permanent access by placement of a catheter with a subcutaneous port through the basilic or cephalic vein into the superior vena cava

**Technical Complications**

- **Infection**
  - When a central venous catheter is used
  - Prevention: catheter dressing, barrier precautions, use of aseptic technique

**Metabolic Complications**

- **Hyperglycemia**
  - Treatment with volume replacement, correction of electrolyte abnormalities, and the administration of insulin

**Initiation of Parenteral Nutrition**

- **15 to 25% dextrose and 3 to 5% crystalline amino acids in sterile conditions**
- **Total Parenteral Nutrition**
  - Periodic infusion of a fat emulsion at a rate equivalent to 10 to 15% of total calories
  - Intraoperative vitamin preparations also should be added to parenteral formulas
  - Vitamin K should be supplemented on a weekly basis
  - Essential trace minerals may be required after prolonged TPN

**Special Formulations**

- **Glutamine**
  - A necessary substrate for nucleotide synthesis in most dividing cells
  - A key amino acid, synthesized within the skeletal muscles and the lung

**Complications of Parenteral Nutrition**

- **Infection**
  - Risk factors: catheter placement, central venous access, infection at the injection site
  - Prevention: strict adherence to barrier precautions, replacing catheters at low risk for infection over a guidewire

**Nutritional supplementation may influence stress-induced inflammatory responses**

- **Intravenous feeding → 1 response to proinflammatory stimuli, e.g., endotoxin**
- **Enteral feedings → 1 systemic inflammation by act as agonists for endogenous neuroendocrine anti-inflammatory (through vagal pathway and signaling via intestinal cholecystokinin receptor)**

**Nutrition-Induced Inflammatory Modulation**

- **Omega-3 Fatty Acids**
  - Nω-3 n-3 fatty acids
  - Improve immune function

- **Nucleotides**
  - mRNA and DNA synthesis
  - Enhance immune function
• Finally finish

• Any QUESTION ??????