Mechanisms of Toxicity

NST110, Toxicology
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Mechanisms of Toxicity

1. Delivery: Site of Exposure to the Target
2. Reaction of the Ultimate Toxicant with the Target Molecule
3. Cellular Dysfunction and Resultant Toxicity
4. Repair or Dysrepair
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1. Delivery: Site of Exposure to the Target

2. Reaction of the Ultimate Toxicant with the Target Molecule

3. Cellular Dysfunction and Resultant Toxicity

4. Repair or Dysrepair
Chemical Factors that Cause Cellular Dysfunction

- **Chemicals that cause DNA adducts** can lead to DNA mutations which can activate cell death pathways; if mutations activate oncogenes or inactivate tumor suppressors, it can lead to uncontrolled cell proliferation and cancer (e.g. benzopyrene)

- **Chemicals that cause protein adducts** can lead to protein dysfunction which can activate cell death pathways; protein adducts can also lead to autoimmunity; if protein adducts activate oncogenes or inactivate tumor suppressors, it can lead to uncontrolled cell proliferation and cancer (e.g. diclofenac glucuronidation metabolite)

- **Chemicals that cause oxidative stress** can oxidize DNA or proteins leading to DNA mutations or protein dysfunction and all of the above. (e.g. benzene, CCl4)

- **Chemicals that specifically interact with protein targets**
  - chemicals that activate or inactivate ion channels can cause widespread cellular dysfunction and cause cell death and many physiological symptoms—Na+, Ca2+, K+ levels are extremely important in neurotransmission, muscle contraction, and nearly every cellular function (e.g. tetrodotoxin closes voltage-gated Na+ channels)
  - Chemicals that inhibit cellular respiration—inhibitors of proteins or enzymes involved in oxygen consumption, fuel utilization, and ATP production will cause energy depletion and cell death (e.g. cyanide inhibits cytochrome c oxidase)
  - Chemicals that inhibit the production of cellular building blocks, e.g. nucleotides, lipids, amino acids (e.g. amanitin from Deathcap mushrooms)
  - Chemicals that inhibit enzymatic processes of bioactive metabolites that alter ion channels and metabolism (e.g. sarin inhibits acetylcholinesterase and elevates acetylcholine levels to active signaling pathways and ion channels)

- **All of the above can also cause inflammation which can lead to cellular dysfunction**
Cellular Dysfunction:
Necrosis versus Apoptosis
Two Forms of Cell Death

1. **Necrosis:** unprogrammed cell death (dangerous)
   
   **A. Passive form of cell death** induced by accidental damage of tissue and does not involve activation of any specific cellular program.
   
   **B.** Early loss of plasma membrane integrity and swelling of the cell body followed by bursting of cell.
   
   **C.** Mitochondria and various cellular processes contain substances that can be damaging to surrounding cells and are released upon bursting and cause inflammation.
   
   **D.** Cells necrotize in response to tissue damage [injury by chemicals and viruses, infection, cancer, inflammation, ischemia (death due to blockage of blood to tissue)].
2. **Apoptosis:** one of the main forms of programmed cell death (not as dangerous to organism as necrosis).

   A. Active form of cell death enabling individual cells to commit suicide.

   B. Caspase-dependent

   C. Dying cells shrink and condense and then fragment, releasing small membrane-bound apoptotic bodies, which are phagocytosed by immune cells (i.e. macrophages).

   D. Intracellular constituents are not released where they might have deleterious effects on neighboring cells.
Necrosis:
- Compromised membrane; Cell swelling
- Cell Lysis; Release of intracellular components
- Physical Trauma
- Complement-mediated Lysis
- Lytic Viral Infection

Apoptosis:
- Cell shrinkage; Chromatin condensation
- Cell Blebbing
- Development
- Tissue Homeostasis
- Cell-mediated Immunity
- Hormone-mediated Atrophy
- Phagocytosis

Mechanisms of Apoptosis

Apoptosis is a cell mechanism used to eliminate cells that contain mutations, are unnecessary, or dangerous to the body.

It is critical to normal embryonic development and to cancer prevention.

Simplified process of apoptosis:

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<tr>
<th>A</th>
<th>Mild convolution</th>
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<tr>
<td>Chromatin</td>
<td>Compaction and segregation</td>
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<td>Condensation of cytoplasm</td>
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<th>B</th>
<th>Nuclear fragmentation</th>
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<td>blebbing</td>
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<td>Cell fragmentation</td>
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Phagocytosis

Aptotic body

Phagocytic cell
Mechanisms of Apoptosis

Phenotypes of apoptosis:
1. Overall shrinkage in volume of the cell and its nucleus
2. Loss of adhesion to neighboring cells
3. Formation of blebs on the cell surface
4. DNA fragmentation: dissection of the chromatin into small fragments
5. Rapid engulfment of the dying cell by phagocytosis

Factors that induce apoptosis:
1. Internal stimuli: abnormalities in DNA
2. External stimuli: removal of growth factors, addition of cytokines (tumor necrosis factor—TNF)

Signal transduction pathways leading to apoptosis:
Two major pathways:
1. Intrinsic pathway (mitochondria-dependent)
2. Extrinsic pathway (mitochondria-independent)
Extrinsic Apoptosis

- The death receptor pathway is activated by external cytokines and is mitochondria-independent.
- The ligands of the death receptors are members of the tumor necrosis factor (TNF) family of proteins, including TNF-alpha, Fas ligand (FasL), TRAIL/Apo2L, Apo3L.
- Binding of ligand to the death receptors results in homotrimerization of the receptors.
- Death receptors contain a death domain in the cytoplasmic region that is required for apoptosis signaling.
Extrinsic Apoptosis

Trimerization of the receptor death domains allows binding and activation of FADD (Fas-associated death domain protein) and formation of death-inducing signaling complex (DISC), which recruits and activates procaspase 8 and 10 to caspase 8 and 10.

Caspases are a family of cysteine-aspartyl-specific proteases that are activated at an early stage of apoptosis and are responsible for triggering most of the changes during apoptosis. Caspases are proteolytically activated and then diffuse into the cytoplasm to cleave target proteins.
Extrinsic Apoptosis

Two major classes of caspases:
1. **Initiator caspases 8,9,10**: initiates the onset of apoptosis by activating the executioner caspases
2. **Initiator caspases 3,6,7**: destroy actual targets in the cell to execute apoptosis

Caspases target:
1. **FAK (focal adhesion kinase)**: inactivation of FAK disrupt cell adhesion, leading to detachment of the apoptotic cell from its neighbors
2. **Lamins**: important component of the nuclear envelope, cleavage of lamins leads to disassembly of the nuclear lamina
3. **Proteins required for cell structure**: actin, intermediate filaments, etc--cleavage of these proteins lead to changes in cell shape and the surface blebbing
4. **Endonuclease CAD**: responsible for chromosome fragmentation. CAD cuts DNA into small fragments. CAD normally binds to an inhibitor protein. Caspases cleaves the inhibitor protein to activate CAD
5. **Enzymes involved in DNA repair**
Extrinsic Apoptosis

- ligands
  - Death receptors
    - FADD/DISC
      - Caspase 8, 10
        - Caspase 3, 6, 7
          - Lamin, CAD, actin, Adhesion molecules, etc.

Diagram:
- FADD/DISC
- DISC
- procaspase-8, procaspase-10
- caspase-8, caspase-10
- procaspase 3
- caspase 3
- apoptosis
Intrinsic apoptosis is mitochondria-dependent and is induced by DNA damage, binding of nuclear receptors by glucocorticoids, heat, radiation, nutrient deprivation, viral infection, hypoxia, and increased intracellular calcium concentration.

Process of Intrinsic apoptosis:
1. Bax forms homo-dimers in the presence of apoptotic signals, opening a channel that translocates cytochrome c from the intermembrane space to the cytoplasm.
2. Bcl2 interferes with Bax function by forming a heterodimer with Bax, closing the channel and inhibiting cytochrome c translocation.
3. In the cytosol, cytochrome c binds to Apaf-1 to form apoptosome.
4. Apoptosome recruits pro-caspase 9 and activates it to caspase 9.
5. Caspase 9 activates executioner caspases 3, 6, and 7.
Summary of Apoptosis

**Ligands**
- Death receptors
  - FADD/DISC
    - Caspase 8, 10
    - Caspase 3, 6, 7
      - Lamin, CAD, actin, adhesion molecules, etc
- Intrinsic apoptosis signals
  - Pro-apoptotic Bcl-2 proteins
    - Cytochrome c translocation
      - Apoptosome (Apaf-1/cyt.c)
        - Caspase 9
Mechanisms of Necrosis

• Cells must synthesize endogenous molecules, assemble macromolecular complexes, membranes, and cell organelles, maintain intracellular environment, and produce energy for operation.

• Agents that disrupt these functions (especially energy-producing function of the mitochondria and protein synthesis) will cause cell death.
Three Primary Metabolic Disorders Jeopardizing Cell Survival:

I. ATP depletion

II. Sustained rise in intracellular Ca^{2+}

III. Overproduction of ROS, RNS

ATP-SYN: ATP synthase
MET: mitochondrial electron transport
NOS: nitric oxide synthase
PARP: poly(ADP-ribose) polymerase
ROS: reactive oxygen species
RNS: reactive nitrogen species
XO: xanthine oxidase
ΔΨm: mitochondrial membrane potential
I. ATP Depletion

ATP plays a central role in cellular maintenance both as a chemical for biosynthesis and as the major source of energy.

1. ATP drives ion transporters such as Na\(^+\)/K\(^+\)-ATPase (plasma membrane), Ca\(^{2+}\) -ATPase (endoplasmic reticulum and plasma membrane) to maintain cellular ion gradients. (most important for necrosis!)

2. Used in biosynthetic reactions (phosphorylation and adenylation)

3. Used for signal transduction regulation (e.g. phosphorylation of receptor tyrosine kinase and kinase pathways)

4. Incorporated into DNA

5. Muscle contraction (actin/myosin interaction) and neurotransmission

6. Polymerization of cytoskeleton (actin and tubule polymerization)

7. Cell division

8. Maintenance of cell morphology
ATP Production in the Mitochondria
Direct Consequences of ATP Depletion

ATP Depletion

compromised ion pumps (e.g., Na/K ATPase and Ca2+-ATPases)

- loss of ionic and volume regulatory controls
- cell swelling (water influx)
  (rise in intracellular Na+)

Ca2+/Na+ levels rise intracellularly and leads to opening of voltage-gated channels that depolarize membranes leading to further Ca2+ and Na+ influx into the cell

- cell lysis

necrosis
Agents That Impair ATP Synthesis

1. Inhibitors of electron transport
   1. Cyanide inhibits cytochrome oxidase
   2. Rotenone inhibits complex I—insecticide that may be an environmental cause of Parkinson’s Disease
   3. Paraquat inhibits complex I—herbicide, but also causes lung hemorrhaging in humans

2. Inhibitors of oxygen delivery
   1. Ischemic agents such as ergot alkaloids, cocaine
   2. Carbon monoxide—displaces oxygen from hemoglobin

3. Inhibitors of ADP phosphorylation - DDT, DIM, phytochemicals

4. Chemicals causing mitochondrial DNA damage - antivirals, chronic ethanol
II. Sustained Rise of Intracellular Ca\textsuperscript{2+}

Ca\textsuperscript{2+} is involved in:

1. signal transduction regulation (i.e. PKC activation by DAG and Ca\textsuperscript{2+}) and exocytosis

2. muscle contraction (actin/myosin interaction)

3. cytoskeletal polymerization (i.e. Ca\textsuperscript{2+} inhibition of actin)

4. neurotransmission (via glutamate receptor Ca\textsuperscript{2+} channel and voltage-gated Ca\textsuperscript{2+} channels) and synaptic plasticity

5. enzyme induction (i.e. citrate and \(\alpha\)-ketoglutarate dehydrogenases from the TCA cycle)

6. Transporters (Ca\textsuperscript{2+}/ATPase, Na/Ca\textsuperscript{2+} exchanger, etc.)
Intracellular Ca\textsuperscript{2+} levels are highly regulated

- The 10,000-fold difference between extracellular and cytosolic Ca\textsuperscript{2+} concentration is maintained by: impermeability of plasma membrane to Ca\textsuperscript{2+} and by transport mechanisms that remove Ca\textsuperscript{2+} from cytoplasm (0.1 \( \mu \)M inside versus 1000 \( \mu \)M outside).

- Ca\textsuperscript{2+} sources are from outside cell or Ca\textsuperscript{2+} stores in ER or mitochondria (as calcium phosphate).
Four mechanisms of calcium elimination:

1. Extracellular $\text{Ca}^{2+}$ ATPase
2. Endoplasmic reticulum $\text{Ca}^{2+}$ ATPase
3. Extracellular $\text{Na}^+$/Ca$^{2+}$ exchanger
4. Mitochondrial $\text{Ca}^{2+}$ uniporter
Excitotoxicity: Consequence of Increased Intracellular Ca$^{2+}$

1. Depletion of energy reserves—decreased mitochondrial ATP production and increased loss of ATP by activation of Ca$^{2+}$-ATPase.

2. Dysfunction of microfilaments—impaired cell motility, disruption in cell morphology, cellular functions

3. Activation of hydrolytic enzymes—disintegration of membranes, proteins, DNA, etc.

4. Generation of ROS/RNS—disintegration of membranes, proteins, DNA, etc.
III. Oxidative Stress

Oxidative stress: imbalance of cellular oxidants and antioxidants in favor of oxidants.
Reactive Oxygen and Nitrogen Species Generation

A. Direct generation of ROS/RNS

a. Xenobiotic bioactivation (i.e. carbon tetrachloride, benzene)
b. Redox cycling (paraquat, MPP+)
c. Transition metals (Fe$^{2+}$, Cu$^{2+}$)
d. Inhibition of mitochondrial electron transport (many phytochemicals)
Reactive Oxygen Stress (ROS) and Reactive Nitrogen Species (RNS)

- Hydrogen peroxide
- Superoxide
- Peroxynitrite
- Nitrosoperoxy carbonate
- Nitrogen dioxide
- Carbonate anion radical
- Hydroxyl radical

\[ \text{Fenton Reaction} \]

\[ \text{Fe(II), Cu(I), Mn(II), Cr(V), Ni(II)} \]

\[ \text{Fe(III), Cu(II), Mn(III), Cr(VI), Ni(III)} \]
B. Indirect generation of ROS/RNS

a. Increased Ca\(^{2+}\) can cause ROS/RNS

i. Activates dehydrogenases in citric acid cycle and *increases electron output (NADH and FADH2)* → leads to an increase in O\(_2^-\) (superoxide) by the e\(^-\) transport chain.

ii. Ca\(^{2+}\) -activated proteases proteolytically convert *xanthine dehydrogenase to xanthine oxidase*, the by-products of which are O\(_2^-\) and H\(_2\)O\(_2\).

iii. Neurons and endothelial cells constitutively express *NOS that is activated by Ca\(^{2+}\) → increase NO production* which reacts with O\(_2^-\) to produce highly reactive ONOO\(^-\) (peroxynitrite).

b. Induction of *CYPs* (i.e. TCDD binding AhR)
Consequences of ROS/RNS

1. ROS can directly oxidize and affect protein function and can mutate DNA leading to cellular dysfunction

2. ROS/RNS oxidatively inactivate Ca$^{2+}$/ATPases and elevate Ca$^{2+}$

3. ROS and RNS also drain ATP reserves:
   a. NO$^-$ is a reversible inhibitor of cytochrome oxidase
   b. ONOO$^-$ irreversibly inactivates complexes I/II/III and aconitase
   c. ROS can disrupt mitochondrial membranes and dissipate the electrochemical gradient needed for ATP synthase.

4. ONOO$^-$ induces DNA single-strand breaks, which activates poly(ADP-ribose) polymerase (PARP)—PARP transfers ADP-ribose moieties from NAD$^+$ to PARP; consumption of NAD$^+$ compromises ATP synthesis

5. Lipid peroxidation, cell swelling, and cell rupture
Lipid Peroxidation

1. Free radicals can initiate peroxidative degradation of lipids by hydrogen abstraction from fatty acids.
2. The lipid radical (L·) formed is converted to the lipid peroxyl radical (LOO·) by oxygen fixation.
3. Lipid hydroperoxide (LOOH) is then formed by hydrogen abstraction from another lipid.
4. Lipid alkoxy radical (LO·) is formed by the Fe(II)-catalyzed Fenton reaction.
5. Fragmentation leads to reactive aldehydes, including the lipid aldehyde and free radicals.

Lipid peroxidation is auto-catalytic.
Cell destruction

Cell osmolarity disruption (transporter disruption)
Cell swelling

**decreased ATP**

- Decreased Ca2+/ATPase
- Decreased mitochondrial potential

- Inactivation of e- transport complexes
- DNA injury
- Decreased NADPH/NADH

**increased Ca2+**

- Decreased Ca2+/ATPase
- Increased mitochondrial e- transport
- Induction of NOS, XO
- Decreased ATP synthase
- Increased e- transport (increased NADH)

**increased ROS**

**increased RNS**

- Lipid peroxidation
- Membrane destruction and/or cell swelling

**cell lysis**
Organophosphate (OP) Nerve Agents

- Organophosphorus (OP) chemical warfare agents inhibit acetylcholinesterase (AChE)
- Under the Nazi regime during World War II, OPs were developed as chemical warfare agents—they are also very easy to manufacture.

![Chemical structures of sarin, VX, and tabun]

Before World War II, chemical warfare was revolutionized by Nazi Germany’s discovery of nerve agents tabun (in 1937) and sarin (in 1939) by Gerhard Schrader, a chemist of IG Farben.

In 1952, researchers in Porton Down, England, invented the VX nerve agent.
Organophosphate (OP) Nerve Agents Act by Irreversibly Inhibiting Acetylcholinesterase

Acetylcholinesterase (AChE) + acetylcholine $\rightarrow$ acetate + choline

AChE active site (serine nucleophile)

AChE irreversibly inhibited by sarin
Acetylcholine binds to **muscarinic ACh receptors** on parasympathetic neurons—controls secretion (salivation, tearing, urination, digestion, defecation), heart rate, breathing

Acetylcholine binds to **nicotinic ACh receptors** on cholinergic neurons—controls memory, motor function, neurotransmission
OPs inhibit AChE, leading to accumulation of acetylcholine at the synapse. Excess acetylcholine hyperstimulates muscarinic ACh receptors leading to excess salivating, vomiting, tearing, urinating, defecating, bronchoconstriction, reduced heart rate, diarrhea. Excess acetylcholine also hyperstimulates nicotinic ACh receptors leading to convulsions and tremors.
MPTP, a contaminant in desmethylprodine (MPPP), an opioid analgesic drug, gave several people in the 1970s and 1980s irreversible Parkinson’s Disease.

In 1976, Barry Kidston, a 23-year old graduate student in Maryland, synthesized MPPP with MPTP as a major contaminant and injected himself —developed full-blown Parkinson’s disease in 3 days—treated with levadopa but died 18 months later from cocaine overdose—autopsy revealed dopaminergic neurodegeneration
MPTP Causes Parkinson’s Disease Through Selective Degeneration of Dopaminergic Neurons in the Substantia Nigra

- **Mechanism of Action:**
  - MPTP crosses the blood brain barrier
  - MPTP gets metabolized to the toxic bioactivated agent MPP+ by monoamine oxidase-B (MAOB) found in glial cells in the brain
  - MPP+ is selectively taken up by dopamine transporters in the brain
  - MPP+ inhibits complex I of the electron-transport chain and causes oxidative stress in dopaminergic neurons to cause neurodegeneration.
  - Over hours to days, patients develop irreversible symptoms of Parkinson’s disease, including tremor, hypokinesia, rigidity, and postural instability
  - Antidote: MAOB inhibitors such as selegiline are used as antidotes to prevent conversion of MPTP to MPP+
Repair Mechanisms

1. DNA repair
2. Protein repair
3. Lipid repair
Oxidized Protein Repair

- Protein disulfides (Prot-SS, Prot1-SS-Prot2), protein sulfenic acids (Prot-SOH) and protein methionine sulfoxides (Prot-Met=O) are reduced by thioredoxin (TR-[SH]2) or methionine sulfoxide reductase; thioredoxin is regenerated by thioredoxin reductase
- Protein glutathione mixed disulfides (Prot-SSF) are reduced by glutaredoxin; glutaredoxin is regenerated by glutathione reductase
• Phospholipid peroxyl radicals (PL-OO·) formed from lipid peroxidation may abstract hydrogens from alpha-tocopherol (TOC-OH), which can be regenerated by glutaredoxin (GRO), which in-turn can be regenerated by glutathione reductase (GR)

• A phospholipase can cleave the fatty acid peroxide (FA-OOH), which can be reduced by glutathione peroxidase (GPX) to give FA-OH; GPX is regenerated by glutathione reductase
Quenching of Oxidative Stress

Detoxification of superoxide anion radical occurs by superoxide dismutase (SOD), followed by glutathione peroxidase (GPO), and catalase (CAT).
DNA Repair Mechanisms

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<tbody>
<tr>
<td>Types of DNA Repair</td>
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<tr>
<td>1. Direct reversal of DNA damage</td>
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<tr>
<td>Alkyltransferases</td>
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<tr>
<td>2. Base excision repair</td>
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<tr>
<td>Glycosylase and AP endonuclease</td>
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<td>3. Nucleotide excision repair</td>
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<tr>
<td>T-T, C-C, C-T repair</td>
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<tr>
<td>“Bulky” adduct repair</td>
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<tr>
<td>4. Double-strand-break repair</td>
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<tr>
<td>Homologous recombination (HR)</td>
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<tr>
<td>Nonhomologous DNA end joining (NHEJ)</td>
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<tr>
<td>5. Mismatch repair</td>
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<td>Repair of deamination of 5-Me cytosine</td>
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<td>Repair of mismatches in DNA due to defective repair, etc.</td>
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SOURCE: Modified from Myles and Sancar (1989) and from Lieber (1998), with permission.
Inflammatory Response
Inflammatory pathway consists of **inducers, sensors, mediators, and target tissues**.

Inducers initiate the inflammatory response and are detected by sensors.

Sensors, like **toll-like receptors (TLRs)** are expressed on specialized sentinel cells such as macrophages, dendritic cells, and mast cells.

- TLRs recognize molecules broadly shared across pathogens (e.g. lipopolysaccharides, double-stranded RNA from viruses, bacterial flagella)
- TLRs also recognize endogenous molecules associated with cell stress (e.g. fibrinogen involved in blood clotting), ATP, heat shock proteins (HSPs), HMBG1 involved in organizing DNA in the nucleosome, and self DNA
When activated, these sensing cells secrete inflammatory mediators including cytokines (e.g. tumor necrosis factor-alpha (TNFα), interleukin-1-beta (IL-1β), and IL-6), chemokines (e.g. CCL2, CXCL8), bioactive amines (e.g. histamine), bradykinin, inflammatory lipids (eicosanoids).

These inflammatory mediators dilate blood vessels, recruit more immune cells, and act on target tissues to eliminate the inflammatory agent, repair the tissue, and elicit changes in their functional states that optimizes their response to noxious conditions.
**TNF Signaling and Effects**

**TNF binds to TNF receptors**, causing the receptor to form a trimer that recruits TRADD, and can activate 3 pathways:

1. **Activation of NF-kB**: TRADD recruits TRAF2 and RIP, TRAF2 recruits protein kinase IKK, which is then activated by RIP. IKK phosphorylates IkBα, which releases NFkB to translocate to the nucleus to act as a transcriptional activator of genes involved in cell survival, proliferation, inflammation, and anti-apoptotic factors.

2. **Activation of MAPK pathways**: TNF induces activation of p38-MAP kinase signaling through activation of ASK1 and MEKK1, eventually leading to the phosphorylation of MKK7 which activates JNK, which is translocated to the nucleus and activates the AP-1 transcription factor to induce cell differentiation and proliferation genes.

3. **Induction of death signaling**: TNF can also induce cell death through TRADD recruiting FAS-associated protein with death domain (FADD), which recruits caspase 8, a protease that activates caspase 3, leading to apoptosis.

Whether a cell undergoes proliferation/inflammation or cell death depends on overall inflammatory environment (other cytokines or ROS).
TNF Signaling and Effects

TNF stimulation leads to:
1. Fever
2. Chemoattractant for neutrophils
3. Stimulates macrophage activation and phagocytosis
4. Production of oxidative stress
5. Production of other inflammatory mediators like eicosanoids
6. Causes insulin resistance
Acute Inflammation Produces ROS and RNS to Eliminate Noxious Insult

Macrophages and some leukocytes recruited to the site of injury undergo a **respiratory burst**, producing free radicals and enzymes to destroy cellular debris and foreign particles.

1. **NAD(P)H oxidase** is activated in macrophages and granulocytes and produces O$_2$$^-$$^-$ from molecular oxygen

   \[
   \text{NAD(P)H} + 2\text{O}_2 \rightarrow \text{NAD(P)}^+ + \text{H}^+ + 2\text{O}_2^- \\
   (\text{O}_2^- \rightarrow \cdot \text{OH via SOD and the Fenton Reaction})
   \]

2. **NOS** is activated in macrophages but not granulocytes by IL-1 and TNF-α

   \[
   \text{L-arginine} + \text{O}_2 \rightarrow \text{L-citrulline} + \cdot \text{NO} \\
   (\cdot \text{NO with O}_2^- \text{ produces ONOO}^- \rightarrow \cdot \text{NO}_2 + \text{CO}_3^-)
   \]
3. **Myeloperoxidase** is discharged by the lysosome into engulfed extracellular spaces, the phagocytic vacuoles

\[
\text{HOOH} + \text{H}^+ + \text{Cl}^- \rightarrow \text{HOH} + \text{HOCl} \quad \text{(hypochloric acid)}
\]
\[
\text{HOCl} + \text{O}_2^- \rightarrow \text{O}_2 + \text{Cl}^- + \text{HO}^•
\]

• All these ROS/RNS are destructive products of inflammatory cells.

• Although these chemicals exhibit antimicrobial activity, **they can damage the adjacent healthy tissues propagating tissue injury.** Thus, **chronic inflammation leads to increased tissue damage.**
Collectively, these inflammatory mediators act to eliminate the inflammatory agent, repair the tissue, and elicit changes in their functional states that optimizes their response to noxious conditions:

1. Dilate blood vessels
2. Recruit more immune cells
3. Destroy noxious agent
4. Undergo an epithelial-to-mesenchymal transition (EMT) to make the basement membrane leakier so immune cells can intravasate into tissues to the site of damage.
5. Secrete growth factors to stimulate cell proliferation to repair damaged tissue
6. After tissue is repaired and noxious agent is gone, inflammatory response is resolved.
Inflammation

A. Under normal conditions, tissue-resident macrophages maintain tissue homeostasis by removing dead cells and other debris and by producing growth factors.

B. Under noxious conditions, the cellular stress response is activated and results in a cell-autonomous adaptation. But it may also involve communication between stressed cells and other cells in the tissue environment, including resident macrophages.

C. If the stress is such that it affects not only individual cells, but the entire tissue, then a tissue-level stress response, or parainflammation, is elicited by the resident macrophages.

D. If the condition is severe, an acute inflammatory response ensues. This is characterized by the recruitment of neutrophils and specialized subsets of monocytes from the circulation that help to protect the host from infection and promote tissue repair and restoration.
Chronic Non-Resolving Inflammation

While inflammation is meant as a defense mechanism against noxious insult, chronic and nonresolving inflammation can cause toxicity and many diseases.

- Tissue fibrosis also occurs from chronic inflammation, e.g. liver fibrosis, lung fibrosis, which can lead to cancer
- Chronic chemical exposures that cause cell death or oxidative stress can lead to nonresolving inflammation
Chronic Non-Resolving Inflammation

Process of Tissue Damage from Non-Resolving Inflammation caused by chronic exposure to toxicant
1. Toxicant causes cellular necrosis
2. Intracellular contents (e.g. ATP, dsDNA, etc) activated TLRs on resident macrophages
3. Macrophage activation leads to secretion of inflammatory cytokines, chemokines, eicosanoids that leads to EMT and leaky basement membrane, vasodilation, recruitment of immune cells, secretion of growth factors
4. Toxicant continues to cause cell death so macrophages continue to get activated and recruited to site of injury
5. Macrophages also secrete TGF-beta, TNF, platelet-derived growth factor (PDGF, insulin growth factor (IGF-1) which stimulates fibroblast proliferation and differentiation leading to excessive formation of an extracellular matrix leading to fibrosis
6. Activated macrophages under respiratory burst and heightened ROS undergo necrosis further exacerbating inflammatory response, fibrosis, cell death, and tissue injury
7. ROS leads to further mutations, activation of cell growth pathways, leading to cancer
8. ROS, macrophages, and cancer cells along with extracellular matrix form a microenvironment that facilitates invasion, angiogenesis, and metastasis
Examples of Non-Resolving Inflammation

A. **Atherosclerosis**—condition where artery wall thickens as a result of the accumulation of fatty materials such as cholesterol and triglycerides—caused largely by the accumulation of fat-and cholesterol-filled macrophages and white blood cells.

B. **Obesity**—abdominal adipose tissue from an obese mouse with necrotic adipocytes surrounded by macrophages.

C. **Rheumatoid Arthritis**—synovium from a human knee infiltrated with lymphocytes, monocytes, and activated fibroblasts.

D. **Pulmonary fibrosis**—collagen is in blue and muscle cells in brown.
Increased circulating free fatty acids (FFA), modified LDL particles, and advanced glycation end products bind to their cognate receptors on macrophages and pancreatic beta-cells, leading to NF-κB activation and production of inflammatory cytokines and chemokines.

Glucose can promote the activation of the NLRP3 inflammasome complex that initiates inflammation.
Adipocytes store excessive nutrient load and progressively become more hypertrophic.

Cell hypertrophy leads to a pro-inflammatory response through hypoxia and ER stress.

Stressed adipocytes produce a wide range of cytokines and chemokines, including TNF, that in-turn promote immune cell accumulation and activation in adipose tissue.
Neuroinflammation is a Hallmark of Neurodegenerative Disease

Inflammation is meant as a defense mechanism against neurotoxic insult. However, chronic non-resolving inflammation can lead to neurodegenerative disorders such as Alzheimer’s and Parkinson’s disease.

Aβ (Alzheimer’s) Bacterial infection Inflammatory chemical

Inflammation leads to reactive microgliosis, which activates microglia. This process can be triggered by neurotoxic factors such as prostaglandins, IL-1β, TNFα, oxidative stress, or glutamate.

Block et al 2007 Nat Rev. Neurosci.; Glass et al., 2010 Cell.
Inflammation in Alzheimer’s Disease

- Amyloid-beta peptide, produced by cleavage of amyloid precursor protein (APP), forms aggregates that activate microglia, in-part by signaling through Toll-like receptors (TLRs) and RAGE.
- These receptors activate NF-κB and AP-1 inducing ROS and inflammatory mediators.
- These inflammatory factors activate astrocytes which amplifies neuroinflammation.
- Collectively, the inflammatory mediators act on cholinergic neurons causing apoptosis and necrosis of neurons.
- Cell death results in release of cellular factors leading to increased microglial activation and increased inflammation.
- Neuroinflammation also upregulates APP and amyloid-beta peptides.
Inflammation in Parkinson’s Disease

Prominent hallmarks of Parkinson’s disease are the loss of dopaminergic neurons in the substantia nigra of the midbrain and the presence of intracellular inclusions containing aggregates of alpha-synuclein protein.

Alpha-synuclein aggregates can also be released from neurons to activate TLRs on microglial cells to initiate inflammatory response.

Chronic and persistent inflammation is sufficient to induce degeneration of dopaminergic neurons.

Pesticides (e.g. paraquat, rotenone) inhibit ATP production through inhibiting complex I in neurons leading to necrosis.

MPTP is a toxicant that causes oxidative stress and is selectively taken up into dopaminergic neurons and inhibits ATP production.
Neuroinflammation and Neurodegenerative Disease

Epidemiologic studies implicate exposure to **herbicides, pesticides, and metals** as risk factors for Parkinson’s disease.

Example: **Rotenone** is a broad-spectrum insecticide that inhibits complex I of the electron-transport chain.

- rotenone belongs to a family of natural cytotoxic compounds extracted from various parts of *Leguminosa* plants.

Behaviorally, rotenone-infused rats exhibit reduced mobility, flexed posture, and in some cases rigidity and even catalepsy. Four weeks after the infusion of rotenone, rats show more than 70% reduction in spontaneous motor activity.
**Paraquat** is a potent herbicide that produces oxidative stress similar to that of MPP⁺

- Paraquat exposure has been epidemiologically linked to Parkinson’s Disease.

- Although paraquat is often associated with massive liver, lung, and kidney damage, patients who have died from paraquat-poisoning also have massive neurodegeneration.
Inflammation and Cancer

- Inflammation acts at all stages of tumorigenesis
- It may contribute to tumor initiation through mutations, genomic instability
- Inflammation activates tissue repair responses, induces proliferation of premalignant cells, and enhances their survival
- Inflammation also stimulates angiogenesis, causes localized immunosuppression, and promotes the formation hospitable microenvironment in which premalignant cells can survive, expand, and accumulate additional mutations
- Inflammation also promotes metastatic spread.
Examples of Environmental “Inflammogens”

- Stress
- Bacterial/viral infections
- Obesity and diabetes
  - Fatty foods
  - Pesticides
  - Metals
  - Gluten
- Trichloroethylene (cleaners)
- Carbon tetrachloride (cleaners, refrigerant)
  - Cigarette smoke
  - Diesel exhaust
  - Physical injury
    - Alcohol
    - Radiation
    - Irritants