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ОБЩАЯ ПАТОФИЗИОЛОГИЯ. ИЗБРАННЫЕ ТЕМЫ

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SELECTED THEMES

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Переиздание дополненного методического пособия предназначеного для подготовки студентов, обучающихся на языке-посреднике в рамках учебного курса патофизиологии. Пособие содержит материал для семинарских и практических занятий по общей патофизиологии.

CONTENTS

CELL INJURY AND DEATH

The cell is a functional and structural unit of the tissue. Cell injury can be defined as the alteration of the cell structure and function that remains and progresses even when the injurious factor (agent) is removed.

Cell injury can be reversible, allowing the cell to recover, or it can be irreversible, causing cell death.

Primary injury involves direct interaction between the cell and the causative factor, in which case the cell is known as target cell.

Secondary injury is due to the expantion of the damage to the neibourhood cells or the whole organism.

Mechanisms of cell injury can involve:

- specific changes, initiated by causative factor,
- nonspecific, universal changes, developing in response to any injurious factor.

Specific changes, initiated by causative factor

Cell damage can occur in many ways. The ways by which cells are specifically injured have been grouped into five categories:

- injury from physical agents,
- radiation injury,
- chemical injury,
- injury from biologic agents,
- injury from nutritional imbalances.

1. Injury From Physical Agents

Physical agents responsible for cell and tissue injury include mechanical forces, extremes of temperature, and electrical forces. They are common causes of injuries due to environmental exposure, occupational and transportation accidents, and physical violence and assault.

Mechanical Forces. Mechanical forces damage cellular membranes and intercellular connections. Injury or trauma due to mechanical forces occurs as the result of body impact with another object. The body or the mass can be in motion, or both can be in motion at the time of impact. These types of injuries split and tear tissue, fracture bones, injure blood vessels, and disrupt blood flow.

Extremes of Temperature. Extremes of heat and cold cause damage to the cell organelles, and its enzyme systems. Exposure to low-intensity heat (43 to 46 C), such as occurs with partial-thickness burns and severe heat stroke, causes acceleration of cell metabolism, inactivation of temperature-sensitive enzymes, and disruption the cell membrane. With more intense heat, coagulation of blood vessels and tissue proteins occurs.

Exposure to cold increases blood viscosity and induces vasoconstriction by direct action on blood vessels and through reflex activity of the sympathetic nervous system.

The resultant decrease in blood flow may lead to hypoxic tissue injury, depending on the degree and duration of cold exposure.

Injury from freezing probably results from a combination of ice crystal formation and vasoconstriction. The decreased blood flow leads to capillary stasis, arteriolar and capillary thrombosis and edema.

Electrical Injuries. Electrical injuries can affect the body through heat generation and disruption of impulses in the exitable tissues. The effect of electricity on the body is mainly determined by:

- its voltage,
- the type of current (i.e., direct or alternating),
- its amperage,
- the resistance of the tissue,
- the pathway of the current,
- the duration of exposure.

Lightning and high-voltage wires that carry several thousand volts produce the most severe damage. Alternating current (AC) is usually more dangerous than direct current (DC) because it causes recharging of cell membranes in the exitable tissues (violent muscle contractions, preventing the person from releasing the electrical source and sometimes resulting in fractures and dislocations).

The resistance to the flow of current in electrical circuits transforms electrical energy into heat. Much of the tissue damage produced by electrical injuries is caused by heat production in tissues that have the highest electrical resistance. Resistance to electrical current varies from the greatest to the least in bone, fat, tendons, skin, muscles, blood, and nerves. Thick, dry skin is more resistant to the flow of electricity than thin, wet skin. It is generally believed that the greater the skin resistance, the greater is the amount of local skin burn, and the less the resistance, the greater are the deep and systemic effects.

In electrical injuries, the body acts as a conductor of the electrical current. The current enters the body from an electrical source, and passes through the body and exits to another conductor. The pathway that a current takes is critical because the electrical energy disrupts impulses in excitable tissues. Current flow through the brain may interrupt impulses from respiratory centers in the brain stem, and current flow through the chest may cause fatal cardiac arrhythmias.

The most severe tissue injury usually occurs at the skin sites where the current enters and leaves the body. After electricity has penetrated the skin, it passes rapidly through the body along the lines of least resistance-through body fluids and nerves. Degeneration of vessel walls may occur, and thrombi may form as current flows along the blood vessels. This can cause extensive muscle and deep tissue injury.

2. Radiation Injury

Electromagnetic radiation comprises a wide spectrum of wave-propagated energy, ranging from ionizing gamma rays to radiofrequency waves. A photon is a particle of radiation energy. Radiation energy above the ultraviolet (UV) range is called ionizing radiation because the photons have enough energy to knock electrons off atoms and molecules. Nonionizing radiation refers to radiation energy at frequencies below that of visible light. UV radiation represents the portion of the spectrum of electromagnetic radiation just above the visible range. It contains increasingly energetic rays that are powerful enough to disrupt intracellular bonds and cause sunburn.

Ionizing radiation affects cells by causing:

- ionization of molecules and atoms in the cell,
- by directly hitting the target molecules in the cell,
- by producing free radicals that interact with critical cell components.

The injurious effects of ionizing radiation vary with the dose, dose rate (a single dose can cause greater injury than divided or fractionated doses), and the differential sensitivity of the exposed tissue to radiation injury.

Most radiation injury is caused by localized irradiation that is used in treatment of cancer. Except for unusual circumstances such as the use of high-dose irradiation that precedes bone marrow transplantation, exposure to whole-body irradiation is rare.

The cell's initial response to radiation injury involves swelling, disruption of the mitochondria and other organelles, alterations in the cell membrane, and marked changes in the nucleus. It can

- immediately kill cells (cytotoxic effect),
- interrupt cell replication (cytostatic effect),
- cause genetic mutations, which may or may not be lethal (mutagenic effect).

Over time, occupational and accidental exposure to ionizing radiation can result in increased risk for the development of various types of cancers, including skin cancers, leukemia, osteogenic sarcomas, and lung cancer.

- Because of the effect on DNA synthesis and interference with mitosis, rapidly dividing cells of the bone marrow and intestine are much more vulnerable to radiation injury than tissues such as bone and skeletal muscle.
- The endothelial cells in blood vessels are particularly sensitive to irradiation. During the immediate postirradiation period, only vessel dilation takes place (e.g., the initial erythema of the skin after radiation therapy). Later or with higher levels of radiation, destructive changes occur in small blood vessels such as the capillaries and venules. Acute reversible necrosis is represented by such disorders as radiation cystitis, dermatitis, and diarrhea from enteritis.
- More persistent damage can be attributed to acute necrosis of tissue cells that are not capable of regeneration and chronic ischemia. Chronic effects of radiation damage are characterized by fibrosis and scarring of tissues and organs in the

irradiated area (e.g., interstitial fibrosis of the heart and lungs after irradiation of the chest).

Ultraviolet Radiation. Ultraviolet radiation causes sunburn and increases the risk for skin cancers. The degree of risk depends on the type of UV rays, the intensity of exposure, and the amount of protective melanin pigment in the skin. Skin damage

induced by UV radiation is caused by reactive oxygen species (ROS) and by damage to melanin-producing processes in the skin. UV radiation also damages DNA, resulting in the formation of pyrimidine dimers Other forms of DNA damage include the production of singlestranded breaks and formation of DNA-protein cross-links.

Nonionizing Radiation. Nonionizing radiation includes infrared light, ultrasound, microwaves, and laser energy. Low-frequency nonionizing radiation is used widely in radar, television, industrial operations (e.g., heating, welding, melting of metals, processing of wood and plastic), household appliances (e.g., microwave ovens), and medical applications (e.g., diathermy).

Unlike ionizing radiation, which can directly break chemical bonds, nonionizing radiation exerts its effects by causing vibration and rotation of atoms and molecules. All of this vibrational and rotational energy is eventually converted to thermal energy.

Isolated cases of skin burns and thermal injury to deeper tissues have occurred in industrial settings and from improperly used household microwave ovens. Injury from these sources is mainly thermal and, because of the deep penetration of the infrared or microwave rays, tends to involve dermal and subcutaneous tissue injury.

3. Chemical Injury

Chemicals capable of damaging cells are everywhere around us. Air and water pollution contain chemicals capable of tissue injury, as does tobacco smoke and some processed or preserved foods. Some of the most damaging chemicals exist in our environment, including gases such as carbon monoxide, insecticides, and trace metals such as lead. Each and every chemical initiates specific injury, they can injure the cell membrane and other cell structures, block enzymatic pathways, coagulate cell proteins, and disrupt the osmotic, ionic, acid-base balance of the cell.

1. Carbon monoxide binds hemoglobin and causes hypoxia.
2. Corrosive substances such as strong acids and bases destroy cells as the substances come into contact with the body.
3. Other chemicals may injure cells in the process of metabolism or elimination. Carbon tetrachloride (CCl_4), for example, causes little damage until it is metabolized by liver enzymes to a highly reactive free radical ($CCl_3\bullet$). Carbon tetrachloride is extremely toxic to liver cells.
4. Lead is a particularly toxic metal. There are innumerable sources of lead in the environment, including flaking paint, lead-contaminated dust and soil, leadcontaminated root vegetables, lead water pipes or soldered joints, newsprint. Lead is absorbed through the gastrointestinal tract or the lungs into the blood and is stored in

bone and eliminated by the kidneys. The major targets of lead toxicity are the red blood cells, the gastrointestinal tract, the kidneys, and the nervous system. Anemia is a cardinal sign of lead toxicity. The gastrointestinal tract is the main source of symptoms in the adult. This is characterized by "lead colic," a severe and poorly localized form of acute abdominal pain. Lead can cause diffuse kidney damage, eventually leading to renal failure. In the nervous system, lead toxicity is characterized by demyelination of cerebral and cerebellar white matter and death of cortical cells. When this occurs in early childhood, it can affect neurobehavioral development and result in lower IQ levels and poorer classroom performance. Peripheral demyelinating neuropathy may occur in adults. The most serious manifestation of lead poisoning is acute encephalopathy.

5. Drugs. Many drugs, prescription drugs and street drugs are capable of directly or indirectly damaging tissues, commonly blood cells, kidney tubules, hepatocytes. For example pathways of drug metabolism can generate toxic drug intermediates or toxic end products. When large amounts of the drug are ingested, the pathway becomes overwhelmed, and toxic metabolites accumulate, causing massive liver necrosis.

Ethyl alcohol can harm the gastric mucosa, liver, pancreas, developing fetus, and other organs. Gastric mucosa can be injured by direct epithelium damage and by increased gastric secretion in combination with decreased production of mucus and bicarbonate. Alcohol causes inflammation of the sphincter of Oddi, leading to retention of hydrolytic enzymes in the pancreatic duct and acini. Alternatively, alcohol may cause decreased tone at the sphincter of Oddi, predisposing to reflux of bile or duodenal contents into the pancreatic duct, leading to parenchymal injury.

4. Injury From Biologic Agents

Biologic agents differ from other injurious agents in that they are able to replicate and can continue to produce their injurious effects. These agents range from submicroscopic viruses to the larger parasites. Biologic agents injure cells by diverse mechanisms.

Viruses enter the cell and become incorporated into its DNA synthetic machinery. Certain viruses cause the cell injury either by the host´s immune response to infected cells or by direct killing of the cell.

Certain bacteria elaborate exotoxins that interfere with cellular production of ATP. Other bacteria, such as the gram-negative bacilli, release endotoxins that initiate inflammation, thrombosis, sepsis.

5. Injury From Nutritional Imbalances

Nutritional excesses and nutritional deficiencies predispose cells to injury. The protein and calorie deficiencies that occur with starvation cause widespread tissue damage. Obesity and diets high in saturated fats predispose persons to atherosclerosis.

The body requires more than 60 organic and inorganic substances in amounts ranging from micrograms to grams. These nutrients include minerals, vitamins, certain

fatty acids, and specific amino acids. Dietary deficiencies can occur in the form of starvation, in which there is a deficiency of all nutrients and vitamins, or because of a selective deficiency of a single nutrient or vitamin.

B12 deficiency anemia, scurvy, beriberi, and pellagra are examples of injury caused by the lack of specific vitamins or minerals.

For example the systemic and cellular concentrations of iron, an essential element, must be tightly regulated to prevent insufficiency or toxic overload. Iron has the capacity to accept and donate electrons readily, interconverting between ferric (Fe^{2+}) and ferrous (Fe^{3+}) forms. This capability makes it a useful component of cytochromes, oxygen-binding molecules (i.e., hemoglobin and myoglobin), and many enzymes. However, iron can also damage tissues by catalyzing the conversion of hydrogen peroxide to free-radical ions that attack cellular membranes, proteins, and DNA. Disorders of iron homeostasis are among the most common diseases of humans.

UNIVERSAL MECHANISMS OF CELL INJURY

There are at least three major mechanisms whereby most injurious agents exert their effects:

- hypoxia and ATP depletion;
- free radical formation;
- disruption of intracellular calcium homeostasis.

Hypoxic Cell Injury

Hypoxia deprives the cell of oxygen and interrupts oxidative metabolism and the generation of ATP. Hypoxia serves as the ultimate cause of cell injury and death in many injuries. For example, toxins from certain microorganisms can interfere with cellular use of oxygen, and a physical agent such as cold can cause severe vasoconstriction and impair blood flow.

Hypoxia literally causes a power failure in the cell, with widespread effects on the cell's functional and structural components. As oxygen tension in the cell falls, oxidative metabolism ceases, and the cell reverts to anaerobic metabolism, using its limited glycogen stores in an attempt to maintain vital cell functions.

Cellular pH falls as lactic acid accumulates in the cell. This reduction in pH can have profound effects on intracellular structures. The nuclear chromatin clumps and myelin figures, which derive from destructive changes in cell membranes and intracellular structures, are seen in the cytoplasm and extracellular spaces.

One of the earliest effects of reduced ATP is acute cellular swelling caused by failure of the energy-dependent sodium/potassium (Na^+/K^+)-ATPase membrane pump, which extrudes sodium from and returns potassium to the cell. With impaired function of this pump, intracellular potassium levels decrease, and sodium and water accumulate in the cell. The movement of fluid and ions into the cell is associated with dilation of the endoplasmic reticulum, increased membrane permeability, and decreased mitochondrial function.

To this point, the cellular changes due to hypoxia are reversible if oxygenation is restored. If the oxygen supply is not restored, however, there is a continued loss of essential enzymes, proteins, and ribonucleic acid through the hyperpermeable membrane of the cell.

Injury to the lysosomal membranes results in leakage of destructive lysosomal enzymes into the cytoplasm and enzymatic digestion of cell components. Leakage of intracellular enzymes through the permeable cell membrane into the extracellular fluid is used as an important clinical indicator of cell injury and death. These enzymes enter the blood and can be measured by laboratory tests.

Free Radical Injury

Many injurious agents exert their damaging effects through a reactive chemical species called a free radical. Free radical injury is rapidly emerging as a final common pathway for tissue damage.

In most atoms, the outer electron orbits are filled with paired electrons moving in opposite directions to balance their spins. A free radical is a highly reactive chemical species arising from an atom that has a single unpaired electron in an outer orbit. In this state, the radical is highly unstable and can enter into reactions with cellular constituents. Moreover, free radicals can establish chain reactions, sometimes thousands of events long. Chain reactions may branch, causing even greater damage. The free radical collides with stable molecules that make up DNA, protein and fat in the cells of the body. This collision allows the free radical to become stable by stealing an electron from the stable molecule. The free radical becomes a balanced molecule once again. The harmless molecule is transformed into a free radical and the cycle begins anew.

Free radical formation is a byproduct of many normal cellular reactions in the body, including energy generation, breakdown of lipids and proteins. Free radical generation is the main mechanism for killing microbes by phagocytic white blood cells.

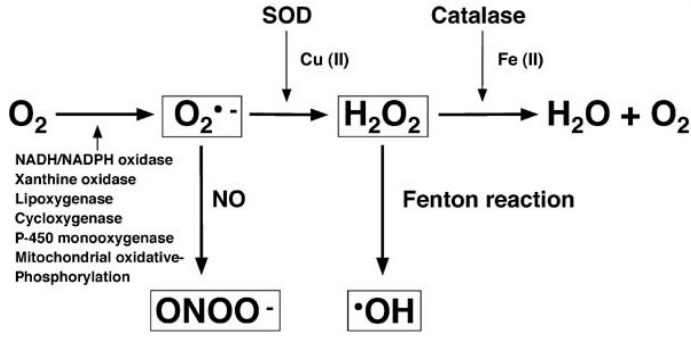


Figure 1. Oxygen derived radicals: superoxide anion (O2·), hydroxyl radical (·OH), peroxynitrite (ONOO-), hydrogen peroxide (H2O2).

SOD – superoxide dismutase.

Molecular oxygen (O2), with its two unpaired outer electrons, is the main source of free radicals (Fig. 1). During the course of normal cellular respiration, molecular oxygen is sequentially reduced in the mitochondria by the addition of four electrons to produce water. During the process, small amounts of partially reduced intermediate species are converted to free radicals.

These reactive species include:

- the superoxide radicals,
- hydrogen peroxide,
- hydroxyl radical.

Transition metals, such as copper and iron, which can accept or donate free electrons during intracellular reactions, are also a source of free radicals. Nitric oxide (NO), an important mediator that is normally synthesized by a variety of cell types, can act as a free radical or be converted into a highly reactive nitrite species.

Uncontrolled free radical production causes damage to cell membranes, cross-linking of cell proteins, inactivation of enzyme systems, damage to the nucleic acids that make up DNA. Oxidative stress occurs when the generation of free radicals in a system exceeds the body's ability to neutralize and eliminate them.

Three types of effects are particularly important in cell injury:

- lipid peroxidation;
- oxidative modification of proteins;
- DNA oxidation.

The superoxide anion and the hydroxyl radical commonly initiate the process of autocatalytic lipid peroxidation. The net result of lipid peroxidation is conversion of unsaturated lipids into polar lipid hydroperoxides, which can cause increased membrane fluidity, including the outer plasma membrane and those of the intracellular organelles; efflux of cytosolic solutes and loss of membrane protein activities. The loss of membrane integrity and increased membrane permeability, results in K+ leakage out of the cell and Na+ and Ca++ influx into the cell occur. Products of oxidized lipids may themselves initiate further oxidative damage which could prove fatal. Thus reactive products such as malondialdehyde and 4-hydroxynonenal may attack amino acid side chains in proteins and cause fragmentation of DNA.

Free radical attack on cell proteins, particularly those of critical enzymes, can interrupt vital processes throughout the cell. Newly synthesized proteins are the most prone to oxidative damage, indicating that complete folding and incorporation into protein complexes confers protection from oxidation-driven degradation. Oxidation-sensitive proteins tend to be associated with particular metabolic pathways or functions. These include energy metabolism, mitochondrial proteins, chaperones and members of the ubiquitin−proteasome system. Vital pathways of energy metabolism are perturbed by protein oxidation. In addition to directly damaging proteins post-

synthesis, certain pro-oxidants cause defects in protein function by targeting the process of mRNA translation.

DNA is an important target of the hydroxyl free radical. Damage can involve: single and double-stranded breaks in DNA, modification of base pairs, cross-links between strands. In most cases, various DNA repair pathways can repair the damage. However, if the damage is extensive, the cell dies. The effects of free radical-mediated DNA changes have also been implicated in aging and malignant transformation of cells.

Antioxidants

Under normal conditions, most cells have chemical mechanisms that protect them from the injurious effects of free radicals – antioxidants. Antioxidants are agents that scavenge ROS, prevent their formation, or repair the damage they cause. These mechanisms commonly break down when the cell is deprived of oxygen or exposed to certain chemical agents, radiation, or other injurious agents.

Scientists continue to investigate the use of free radical scavengers to protect against cell injury during periods when protective cellular mechanisms are impaired. Defenses against free radicals include vitamins E and C. Vitamin E is the major lipid-soluble antioxidant present in all cellular membranes. Vitamin C is an important watersoluble cytosolic chain-breaking antioxidant; it acts directly with superoxide and singlet oxygen radicals. Several enzymes – superoxide dismutase, glutathione peroxidases, catalase are main intracellular antioxidants; metal-binding proteins transferring, ceruloplasmin, albumin also are known as antioxidants.

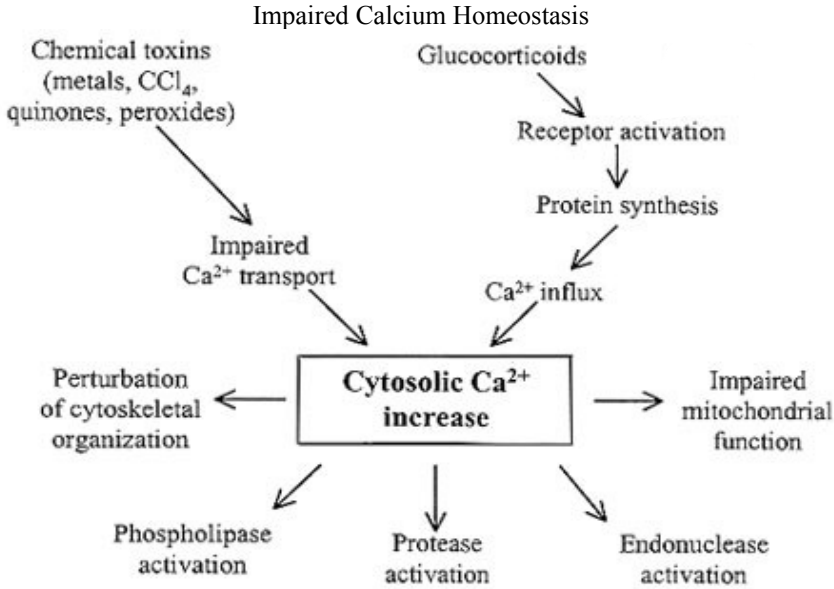


Figure 2. Damaging effects of the increased intracellular calcium level

Calcium functions as a messenger for the release of many intracellular enzymes. Normally, intracellular calcium levels are kept extremely low compared with extracellular levels.

These low intracellular levels are maintained by energy dependent membrane-associated calcium/magnesium ($Ca2^+$/$Mg2^+$)-ATPase exchange systems. Ischemia, certain toxins, free radicals lead to an increase in cytosolic calcium because of increased influx across the permeable cell membrane and the release of calcium stored in the mitochondria and endoplasmic reticulum. The increased calcium level activates a number of enzymes with potentially damaging effects (Fig. 2).

The enzymes include:

- the phospholipases responsible for damaging the cell membrane,
- proteases that damage the cytoskeleton and membrane proteins,
- ATPases that break down ATP and hasten its depletion,
- endonucleases that fragment chromatin.

Reversible Cell Injury

Reversible cell injury, although impairing cell function, does not result in cell death. Two patterns of reversible cell injury can be observed under the microscope: cellular swelling and fatty change.

Cellular swelling occurs with impairment of the energy-dependent Na^+/K^+-ATPase membrane pump, usually as the result of hypoxic cell injury. Fatty changes are linked to intracellular accumulation of fat. When fatty changes occur, small vacuoles of fat disperse throughout the cytoplasm. These fatty changes may occur because injured cells are unable to metabolize the fat properly. The liver, where most fats are synthesized and metabolized, is particularly susceptible to fatty change, but fatty changes may also occur in the kidney, the heart, and other organs.

Cell adaptaton to the injury includes:

- Decrease of the specific cell functional activity.
  - Activation of anaerobic ATP resynthesis in the cytosol and aerobic respiration in the intact mitochondria.
- Activation of the antioxidative systems.
  - Activation of buffer systems.
  - Upon sensing DNA damage, cell cycle checkpoints are activated to arrest cell cycle progression to allow time for repair before the damage is passed on to daughter cells. The DNA damage response leads to induction of transcriptional programs, enhancement of DNA repair pathways: exision of the altered sites in the DNA molecule, restoration of the native DNA fragments. When the level of DNA damage is severe, apoptosis is initiated.

Manifestations of the adaptation to the cell injury at the tissue level are regeneration, hypertrophy, hyperplasia.

CELL DEATH

Cell destruction and removal can involve one of two mechanisms:

- apoptosis, programmed cell removal, which is designed to remove injured or worn-out cells,
- necrosis, which occurs in severely damaged cells (Fig. 3).

Programmed Cell Death – Apoptosis.

The process was first described in 1972. In each cell line, the control of cell number is regulated by a balance of cell proliferation and cell death. Apoptotic cell death involves controlled cell destruction and is involved in normal cell deletion and renewal. Apoptosis, from Greek "apo" for "apart" and "ptosis" for "fallen," means fallen apart. For example, blood cells that undergo constant renewal from progenitor cells in the bone marrow are removed by apoptotic cell death.

Apoptotic cell death, which is equated with cell suicide, eliminates cells that are worn out, have been produced in excess, have developed improperly, or have genetic damage. In normal cell turnover, this process provides the space needed for cell replacement.

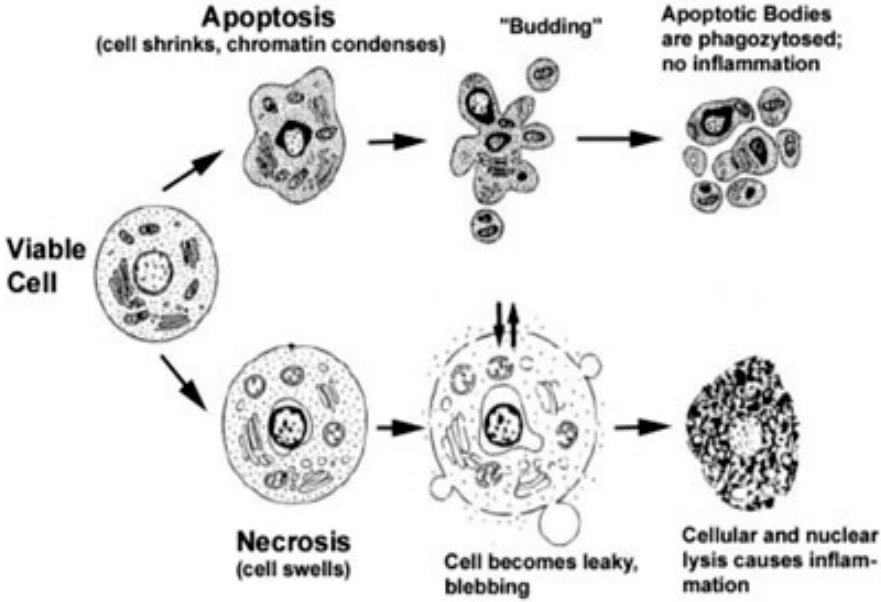


Figure 3. Necrosis and apoptosis

Apoptotic cell death is characterized by controlled energy dependent autodigestion of cell components. Cells appear to initiate their own death through the activation of endogenous enzymes-caspases. This result in cell shrinkage brought about by:

- disruption of the cytoskeleton,
- condensation of the cytoplasmic organelles,
- disruption and clumping of nuclear DNA,
- distinctive wrinkling, "budding" of the cell membrane.

As the cell shrinks, the nucleus breaks into spheres, and the cell eventually divides into membrane-covered fragments – apoptotic bodies. Membrane changes occur during the process, signaling surrounding phagocyte cells to engulf the apoptotic cell parts and complete the degradation process.

Apoptosis is thought to be responsible for several normal physiologic processes, including:

- programmed destruction of cells during embryonic development,
- hormonedependent involution of tissues,
- death of immune cells,
- cell removal by cytotoxic T cells,
- cell death in proliferating cell populations.

During embryogenesis, in the development of a number of organs such as the heart, which begins as a single pulsating tube and is gradually modified to become a four-chambered pump, apoptotic cell death allows for the next stage of organ development. It also separates the webbed fingers and toes of the developing embryo.

Apoptotic cell death occurs in the hormone-dependent involution of endometrial cells during the menstrual cycle and in the regression of breast tissue after the period of breast-feeding. The control of immune cell numbers and destruction of autoreactive T cells in the thymus have been credited to apoptosis. Cytotoxic T cells and natural killer cells are thought to destroy target cells by inducing apoptotic cell death.

Apoptotic cell death can be triggered by various death stimuli. These death stimuli can activate defined cellular death machineries that can best be described as extrinsic and intrinsic.

- In the extrinsic pathway, which is also known as the receptor-mediated, proteins – members of the tumor necrosis factor (TNF) superfamily bind to cell-surface «death receptors». Ligation of these receptors initiates the catalytic activity of caspase 8, a central mediator of apoptosis.

- In the intrinsic pathway, which is also known as the mitochondria-mediated, increased intracellular ROS, DNA damage, the unfolded protein response, and the deprivation of growth factors lead to increased mitochondrial permeability, promoting the release of proapoptotic proteins (cytochrome C) and activation of caspase 9.

Activated caspase 8 (death-receptor pathway) and caspase 9 (mitochondrial pathway) in turn mobilize caspases 3, 6, and 7, proteases that herald destruction of the cell by cleaving numerous proteins and activating DNases.

Apoptosis appears to be linked to several pathologic processes.

For example, suppression of apoptosis may be a determinant in the growth of cancers. Certain oncogenes and suppressor genes involved in the development of cancer play an active role in stimulation or suppression of apoptosis.

Apoptosis is also thought to be involved in the cell death associated with certain viral infections. In hepatitis B and C, the virus seems to sensitize the hepatocytes to apoptosis.

Apoptosis may also be involved in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease. The loss of cells in these disorders does not induce inflammation; although the initiating event is unknown, apoptosis appears to be the mechanism of cell death.

Apoptotic cell death may be caused by a variety of injurious agents and is usually activated at stressor doses lower than those leading to necrotic cell killing:

- mild thermal injury,
- low dose radiation injury,
- mild hypoxia.

Several mechanisms are involved in initiating cell death by apoptosis:

- as in the case of endometrial changes that occur during the menstrual cycle, the process can be triggered by the addition or withdrawal of hormones;
- injured cells may induce apoptotic cell death through increased cytoplasmic calcium, which leads to activation of nuclear enzymes endonucleases that break down DNA;
- cell surface signaling or receptor activation by mediators –TNF, IL-1, IL-10 appears to be the influencing force;
- pro-oxidants induce the intrinsic apoptosis pathway, involving the release of pro-apoptotic factors from damaged mitochondria.

Necrosis

Necrosis refers to cell death in an organ or tissue that is still part of a living person, traditionally considered an accidental form of cell death. Necrosis usually results from metabolic failure that has coincided with rapid depletion of ATP. It classically occurs in severe ischemia, mechanical force, heat, cold, and membrane-permeabilizing toxins. Many insults induce apoptosis at lower doses and necrosis at higher doses. Necrosis differs from apoptosis in that it involves unregulated enzymatic digestion of cell components. The compromise of organellar membranes allows proteolytic enzymes to escape from lysosomes, enter the cytosol, and cause cell destruction. The loss of cell membrane integrity allows:

- an influx of extracellular ions and fluid, with resultant swelling of the cell and its organelles;
- uncontrolled release of the products of cell death into the intracellular space, and initiation of the inflammatory response.

In contrast to apoptosis, which functions in removing cells so that new cells can replace them, necrosis often interferes with cell replacement and tissue regeneration. The dissolution of the necrotic cell or tissue can follow several paths.

The cell can undergo:

- liquefaction (i.e., liquefaction necrosis); it occurs when some of the cells die but their catalytic enzymes are not destroyed, an example is the softening of the center of an abscess with discharge of its contents;
- transformation to a gray, firm mass (i.e., coagulation necrosis); during coagulation necrosis, acidosis develops and denatures the enzymatic and structural proteins of the cell, it is characteristic of hypoxic injury and is seen in infarcted areas;
- convertion to a cheesy material by infiltration of fatlike substances (i.e., caseous necrosis). Caseous necrosis is a distinctive form of coagulation necrosis in which the dead cells persist indefinitely as soft, cheeselike debris, it is most commonly found in the center of tuberculosis granulomas, or tubercles, and is thought to result from immune mechanisms.

GENERAL MECHANISMS OF HYPOXIA

Hypoxia can be defined as a typical pathologic process characterized by a decrease in oxidative metabolism, leading to the ATP depletion. Hypoxia is a decrease in tissue oxygen supply below normal levels. The definition of anoxia varies somewhat more than that of hypoxia. The term generally refers to a more severe state of oxygen deficiency and generally carries an implication of irreversible tissue damage. Hypoxemia refers to deficient oxygen in the blood, specifically in the arterial blood. Hypoxia denotes oxygen deficiency at the mitochondrial sites due to insufficient delivery of oxygen (low P_aO_2) or inability to utilise oxygen (normal P_aO_2).

Hypoxia can result from:

1. An inadequate amount of oxygen in the air, so called exogenous hypoxia. Hypobaric form results from breathing air at subnormal pressure on ascent to altitude in the mountains and normobaric form that can occur in closed spaces or when oxygen is displaced by other gases (CO, anesthetic gases). Exogenous hypoxia is characterized by a primary decrease in the partial alveolar oxygen pressure (PAO2), resulting in decrease in the partial arterial oxygen pressure (PaO_2).
2. Respiratory diseases – respiratory hypoxia, which may be induced by alveolar hypoventilation, impaired blood circulation (perfusion) in lungs, ventilation to perfusion mismatch, lung diffuse capacity disorders. It is characterized by low oxygen pressure in the alveoli (PAO2), reduced oxygen pressure in the arterial blood (PaO_2).

3. Cardiovascular diseases (ischemia) – circulatory hypoxia, which is caused by decreased blood flow due to circulatory disorders. It may be total as with myocardial infarction and shock affecting the oxygen content of the blood and all of the cells in the body or local as with thrombosis affecting blood flow through small numbers of blood vessels and producing local tissue injury. Infarction (i.e., tissue death) occurs when an artery supplying an organ or part of the body becomes occluded and no other source of blood supply exists. An artery may be occluded by an embolus, a thrombus, disease of the arterial wall, or pressure from outside the vessel. Ischemia is characterized by impaired oxygen delivery and impaired removal of metabolic end products such as lactic acid. The arterial oxygen tension (PaO_2) and content (CaO_2) may be normal, but the venous oxygen is reduced.

4. Anemic hypoxia: is due to a decreased concentration of functional hemoglobin (abnormal hemoglobin affinity to oxygen) or a reduced amount of hemoglobin and number of red blood cells – failure of blood production (deficitic, aplastic anemias), excessive blood turnover – blood loss, hemolysis. Hemoglobin oxygen binding capacity is disturbed by carbon monoxide (CO), which binds to the same side of hemoglobin molecule as oxygen, but its binding affinity is 200 times higher. Some drugs and foods such as nitrites, phenacetin can produce methemoglobin uncapable of carrying oxygen. It is characterized by reduction of the oxygen carrying capacity of the blood. Arterial oxygen tension (PaO_2) may be normal, but oxygen content (CaO_2) is reduced.

5. Inability of the cells to use oxygen – histotoxic hypoxia. It is due to respiratory enzymes deficit or mitochondria dysfunction. In hypermetabolic states, the cells may require more oxygen than can be supplied by normal respiratory function and oxygen transport. Oxygen delivery remains normal, but the venous oxygen tension and content are high.

Hypoxia typically involve a primary or initiating event and a compensatory or adaptive state that results from homeostatic mechanisms that attempt to correct or prevent large changes in oxygen delivery.

Adaptation at the cellular level

All nucleated cells in the body sense and respond to hypoxia. Under conditions of reduced oxygen availability, hypoxia-inducible factor 1 (HIF-1) regulates the expression of genes that mediate adaptive responses. HIFs represent the link between oxygen sensors and effectors at the cellular, local, and systemic level.

HIF-1 activity was detected in various cell lines cultured under hypoxic conditions, providing the evidence that HIF-1 is part of a widespread oxygen sensing and signal transduction mechanism. Functional analyses revealed that HIF-1 is a phosphorylation-dependent and redox-sensitive protein, contacting DNA in the major groove. Under hypoxic conditions, HIF-1α is stabilized, undergoes modifications, translocates into the nucleus, recruits cofactors, and activates gene expression.

HIF-1 target genes involved in iron metabolism. Iron is required for heme formation and is the most common limiting factor in erythropoiesis. Hypoxia was found to increase the expression of transferrin, probably to enhance the iron transport to erythroid tissues. The transferrin receptor is a hypoxia-inducible HIF-1 target gene, enabling cellular transferrin uptake. Ceruloplasmin was shown to be a HIF-1 target gene. Ceruloplasmin, also known as a ferroxidase, is required to oxidize ferrous to ferric iron. Because only ferric iron can be bound by transferrin, hypoxic ceruloplasmin induction is likely to support iron supply to erythroid tissues.

HIF-1 target genes involved in vascular biology. The vascular system underlies tight oxygen-regulated control. VEGF (vascular endothelial growth factor) is the most prominent HIF-1 target gene involved in vascular biology. HIF-1 seems to regulate flt-1, one of the receptors for VEGF. Recently, an endocrine-gland-derived VEGF (EG-VEGF) was reported that might be a HIF-1 target. The induction of angiogenesis leads to an increase in the vascular density and hence a decrease in the oxygen diffusion distance. However, local blood flow under pathophysiological conditions is controlled by modulation of the vascular tone through production of NO (inducible nitric oxide synthase), endothelin 1, adrenomedullin, or activation of the α_{1B}-adrenergic receptor, all of which involve HIF-1 target genes, too.

HIF-1 target genes involved in glucose metabolism. Under conditions of limited oxygen supply, anaerobic glycolysis becomes the predominant form of cellular ATP generation. Many genes involved in glucose uptake and glycolysis were identified as HIF-1 target genes. Enhanced lactate production and hence a decrease in pH results from the increase in anaerobic glycolysis, potentially limiting this source of ATP despite sufficient glucose supply. Transmembrane carbonic anhydrases were reported to be HIF-1 target genes. Carbonic anhydrases regulate the pH by converting protons and bicarbonate to carbon dioxide, which can be taken up by erythrocytes for transportation to the lung.

Adaptation at the body level

Manifestations of acute hypoxia are due to ventilatory as well as cardiovascular compensatory responses, mediated by hypoxic chemoreflex stimulation:

- hyperventilation,
- tachycardia,
- vascular effects − vasodilation of coronary and brain vessels; vasoconstriction in skin, skeletal muscle and the splanchnic area.

Adjustments of respiration and circulation in response to alterations in the levels of oxygen, carbon dioxide and hydrogen ions in the body fluids are mediated by two distinct chemoreceptive elements, situated peripherally and centrally(Fig. 4).

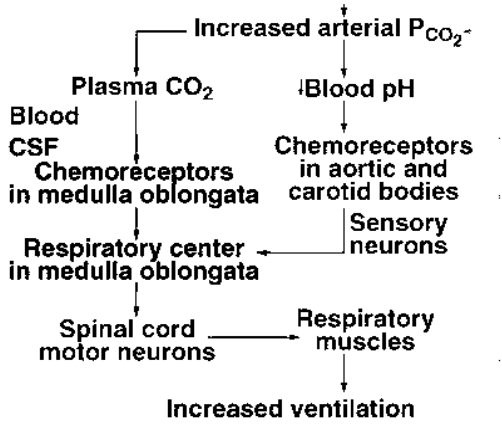


Figure 4. Chemoreflex stimulation of breating rate.

Peripheral arterial chemoreceptors are located in the carotid and aortic bodies. The carotid receptors, and aortic receptors, are essential for the immediate ventilatory and arterial pressure increases during acute hypoxaemia, and also make an important contribution to respiratory compensation for acute disturbances of acid-base balance. The carotid bodies function as sensors of arterial oxygenation (PaO_2). This response is most marked below 60 mm Hg. An increase in the $PaCO_2$ or a fall in arterial pH will potentiate the response of the carotid body to a decrease in the PaO_2.

Central chemoreceptive elements respond to changes in the hydrogen ion concentration, and are chiefly responsible for ventilatory and circulatory adjustments during hypercapnia – changes in $PaCO_2$ and chronic disturbances of acid-base balance. The central or medullary chemoreceptors lie within 200 μm of the anterolateral surfaces of the medulla and are separate from the neurons that generate the respiratory rhythm. Medullary chemoreceptors respond to changes in the hydrogen ion concentration of their extracellular fluid. The blood-brain barrier permits free diffusion of carbon dioxide but not hydrogen ions. Carbon dioxide is hydrated to carbonic acid, which ionizes and lowers the cerebrospinal fluid pH. Central chemoreceptors are not stimulated by a fall in PaO_2; in fact, they are depressed by hypoxia.

Decompensated hypoxia causes structural and functional changes in certain tissues. The actual time necessary to produce irreversible cell damage depends on the degree of oxygen deprivation and the metabolic needs of the cell.

Highly vulnerable to hypoxia: cardiac myocytes, neurons, proximal tubule epithelium, endothelial cells, pneumocytes.

Relatively resistant to hypoxia: adipocytes, bones, skin.

Well-differentiated cells, such as those in the heart, brain, and kidneys, require large amounts of oxygen to provide energy for their special functions. Brain cells, for example, begin to undergo permanent damage after 4 to 6 minutes of oxygen deprivation. A thin margin can exist between the time involved in reversible and irreversible cell damage. One study found that the epithelial cells of the proximal tubule of the kidney in the rat could survive 20 but not 30 minutes of ischemia. Within

60 seconds after coronary artery occlusion, myocardial oxygen tension in the affected cells falls to zero, cardiac stores of high-energy phosphates are rapidly depleted and the cells shift to anaerobic metabolism with consequent lactic acid production.

Decompensated hypoxia is expressed by:

- Redistribution of the blood from cutaneous tissues resulting in pale skin, mucous membranes.
- Increased amount of desaturated hemoglobin results in cyanosis of the skin,

mucosa and nail beds.

- Cardiac hypoxia can be manifested by decreased force of contraction and dysrhythmias.
- Brain hypoxia results in: headache, fatigue, slowed reflexes, slurred speech,

unconsciousness.

- Coma and death can occur in minutes or hours.

Physiological adapting responses to chronic hypoxia at the tissue level include

- hypertrophy − ventricular hypertrophy, respiratory muscle hypertrophy,
- hyperplasia − hyperplasia of the bone marrow, vascular remodeling − angiogenesis leading to an increase in the vascular density and a decrease in the oxygen diffusion distance.

Symptoms and signs of chronic hypoxia include:

- Complaints of difficult breathing or dyspnea with exertion; with severe hypoxia, dyspnea may be present even at rest. Hyperventilation is due to the hypoxic chemoreflex stimulation that persists for a lifetime, Cheyne-Stokes breathing can occur.
- Tachycardia also appears to result from the hypoxic chemo- and baroreflex stimulation and increases oxygen delivery by increasing cardiac output, as hypoxia progresses hypertrophy of cardic muscle develops.
- Pale skin occures with vasoconstriction, high concentration of deoxygenated hemoglobin in the capillaries leads to cyanosis of the skin, mucosa and nail beds.
- Polycytemia is induced by increased production of erythropoetin and hyperplasia of the bone marrow.
- Muscle weakness and listlessness may result from alterations in muscle metabolism.
- A person often complains of weakness, fatigue, general malaise, and a dull headache, as hypoxia progresses, the level of consciousness declines, and stupor and coma can develop.

BLOOD GASES

Blood gases give us three different oxygen values: the partial pressure of oxygen (PaO_2), oxygen saturation (SaO_2), oxygen content (CaO_2).

Oxygen pressure - PaO_2

The partial pressure of oxygen in the plasma phase of arterial blood, is registered by an electrode that senses randomly-moving, dissolved oxygen molecules. Oxygen molecules dissolved in plasma (i.e., not bound to hemoglobin) are free to impinge on the measuring oxygen electrode. This "impingement" of free O_2 molecules is reflected as the partial pressure of oxygen; if the sample being tested is arterial blood, then it is the PaO_2.

The amount of dissolved oxygen in the plasma phase - and hence the PaO_2 - is determined by alveolar PO_2 and lung architecture only, and is unrelated to anything about hemoglobin.

Since PaO_2 reflects only free oxygen molecules dissolved in plasma and not those bound to hemoglobin, PaO_2 cannot tell us "how much" oxygen is in the blood; for that you need to know how much oxygen is also bound to hemoglobin, information given by the SaO_2 and hemoglobin content. Oxygen molecules that pass through the thin alveolar-capillary membrane enter the plasma phase as dissolved (free) molecules; most of these molecules quickly enter the red blood cell and bind with hemoglobin. There is a dynamic equilibrium between the freely dissolved and the hemoglobin-bound oxygen molecules. The number of O_2 molecules dissolved in plasma determines, along with other factors, how many molecules will bind to hemoglobin, the more dissolved molecules there are (i.e., the greater the PaO_2) the more will bind to available hemoglobin; thus SaO_2 always depends, to a large degree, on the concentration of dissolved oxygen molecules (PaO_2).

The most common physiologic disturbance of lung architecture, and hence of a reduced PaO_2, is ventilation-perfusion (V-Q) imbalance. Less common causes are reduced alveolar ventilation, diffusion block, and anatomic right to left shunting of blood.

Oxygen saturation - SaO_2

Binding sites for oxygen are the heme groups, the Fe^{++}-porphyrin portions of the hemoglobin molecule. There are four heme sites, and hence four oxygen binding sites, per hemoglobin molecule. If there is no interference (as from carbon monoxide, for example), the free O_2 molecules bind to these sites with great avidity. Heme sites occupied by oxygen molecules are said to be "saturated" with oxygen.

The percentage of all the available heme binding sites saturated with oxygen is the hemoglobin oxygen saturation (in arterial blood, the SaO_2). The total percentage of sites actually bound with O_2 is constant for a given set of conditions, and is the 'saturation of blood with oxygen'. This is called SvO_2 and SaO_2 in the venous and arterial circulations, respectively (the normal values are 75% and 97%).

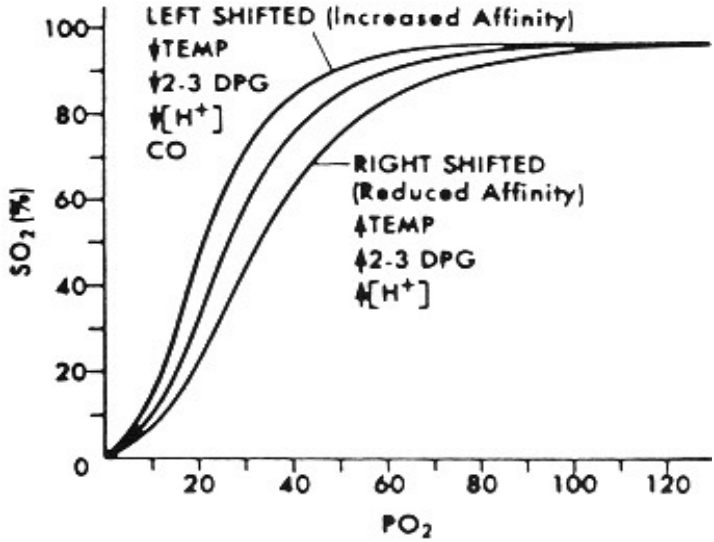


Figure 5. Oxygen dissociation curve

A SaO_2 of 97% simply means that of every 100 hemoglobin binding sites, 97 are occupied with an oxygen molecule and the other three are either bound to something else or are unbound. Note that SaO_2 alone doesn't reveal how much oxygen is in the blood; for that we also need to know the hemoglobin content. SaO_2 is determined mainly by PaO_2. The relationship between the two variables is the familiar oxygen dissociation curve (Fig. 4). At low oxygen pressures there is relatively little increase in SaO_2 for a given change in PaO_2. Above a PaO_2 of 20 mm Hg, the rate of change of SaO_2 increases markedly, then slows again beyond a PaO_2 of 60 mm Hg.

PaO_2 is the most important (but not the only) determinant of SaO_2. Other determinants of SaO_2 for a given PaO_2 are conditions that shift the position of the oxygen dissociation curve left or right, such as temperature, pH, $PaCO_2$ and level of 2,3-DPG in the blood (Fig. 5).

SaO_2 is unaffected by the hemoglobin content, so anemia does not lower SaO_2. The more hemoglobin, the more oxygen molecules will be bound in a given volume of blood, but the percentage of available hemoglobin sites bound to oxygen (the SaO_2) depends only on the PaO_2 and curve-shifting factors. Thus, a patient can have a normal PaO_2 and SaO_2, but still have a low CaO_2 (e.g., with anemia).

Oxygen content - CaO_2

Tissues need a requisite amount of O_2 molecules for metabolism. Neither the PaO_2 nor the SaO_2 provide information on the number of oxygen molecules, i.e., of how much oxygen is in the blood. Of the three values used for assessing blood oxygen levels, how much is provided only by the oxygen content, CaO_2. This is because CaO_2 is the only value that incorporates the hemoglobin content. CaO_2, unlike either

PaO_2 or SaO_2, directly reflects the total number of oxygen molecules in arterial blood, both bound and unbound to hemoglobin. 1g of hemoglobin, completely saturated with oxygen, binds 1,34 ml of oxygen, the solubility of oxygen in plasma is 0.003 ml O_2/dl plasma/mm Hg. The CaO_2 component bound to hemoglobin can be calculated by (Hb x 1.34 x SaO_2) and the dissolved component by (0.003 x PaO_2). This equation can be used to calculate oxygen content of any blood or plasma sample. Thus, oxygen content can be measured directly or calculated by the oxygen content equation:

$$CaO_2 = Hb\ (g/l) \times 1.34\ (ml\ O_2/g\ Hb) \times SaO_2 + PaO_2 \times 0.03\ (ml\ O_2/mm\ Hg/l)$$

In contrast to the other two variables, CaO_2 depends on the hemoglobin content and is directly related to it; other determinants of CaO_2 are the SaO_2 (in turn dependent on PaO_2 and position of the oxygen dissociation curve), and the amount of dissolved oxygen (the PaO_2). Since the dissolved oxygen contributes minimally to CaO_2 under physiologic conditions, CaO_2 is determined almost entirely by hemoglobin content and SaO_2, and is related linearly to either variable.

Normal CaO_2 ranges from 160 to 220 ml O_2/l. Because PaO_2 and/or SaO_2 can be normal in certain conditions associated with hypoxemia, one should always make sure CaO_2 is adequate when assessing oxygenation. About 98% of the normal O_2 content is carried bound to hemoglobin.

Answer the following questions:

1. At 10 a.m. a patient has a PaO_2 of 85 mm Hg, and SaO_2 of 98%, and a hemoglobin of 140 g/l. At 10:05 a.m. she suffers a severe hemolytic reaction that suddenly leaves her with a hemoglobin of 70 g/l. Assuming no lung disease occurs from the hemolytic reaction, what will be her new PaO_2, SaO_2, and CaO_2?

a) PaO_2 unchanged, SaO_2 unchanged, CaO_2 unchanged b) PaO_2 unchanged, SaO_2 unchanged, CaO_2 reduced c) PaO_2 reduced, SaO_2 unchanged, CaO_2 reduced

d) PaO_2 reduced, SaO_2 reduced, CaO_2 reduced.

2. Which patient is more hypoxemic, and why?

Patient A: pH 7.48, $PaCO_2$ 34 mm Hg, PaO_2 85 mm Hg, SaO_2 95%, Hemoglobin 70 gm/l.

Patient B: pH 7.32, $PaCO_2$ 74 mm Hg, PaO_2 55 mm Hg, SaO_2 85%, Hemoglobin 150 gm/l.

3. State which of the following situations would be expected to lower PaO_2.

a) anemia

b) carbon monoxide toxicity

c) an abnormal hemoglobin that holds oxygen with half the affinity of normal hemoglobin

d) an abnormal hemoglobin that holds oxygen with twice the affinity of normal hemoglobin

e) lung disease with intra-pulmonary shunting.

ACID-BASE DISORDERS

- An acid is a molecule that can release a hydrogen ion (H^+).
- A base is a molecule that can accept or combine with a hydrogen ion.

When an acid (HA) is added to water, it dissociates reversibly to form H^+ and anions (A^-); for example, HA= H^+ + A^-. The degree to which an acid dissociates and acts as a H^+ ion donor determines whether it is a strong or weak acid. Strong acids, such as sulfuric acid, dissociate completely; weak acids, such as acetic acid, dissociate only to a limited extent. The same is true of a base and its ability to dissociate and accept an H^+ ion.

Most of the body's acids and bases are weak acids and bases; the most important are carbonic acid (H_2CO_3), which is a weak acid derived from carbon dioxide (CO_2), and bicarbonate (HCO_3^-), which is a weak base.

The concentration of the H^+ ion in body fluids is low compared with other ions. For example, the sodium ion (Na+) is present at a concentration approximately 1 million times that of the H^+ ion.

Because of its low concentration in body fluids, the H+ ion is commonly expressed in terms of pH.

Specifically, pH represents the negative logarithm (p) of the H^+ ion concentration in milliequivalents per liter; a pH value of 7.0 implies an H^+ ion concentration of 10^-7 (0.0000001) equivalents per liter (mEq/L). The pH is inversely related to the H^+ ion concentration; a low pH indicates a high concentration of H^+ ions, and a high pH indicates a low concentration of H^+ ions.

Metabolic activities of the body require the precise regulation of acid-base balance, which is reflected in the pH of extracellular fluids. Ionic balance (K+, Ca++, Na+, membrane excitability), enzymatic activity (chemical reactions) depend on acid-base balance being regulated within a narrow physiologic range to function in an optimal way. Normally, the concentration of body acids and bases is regulated so that the pH of extracellular body fluids is maintained within a very narrow range of 7.35 to 7.45. This balance is maintained through mechanisms that generate buffer and eliminate acids and bases.

METABOLIC ACID AND BICARBONATE PRODUCTION

Acids are continuously generated as byproducts of metabolic processes. Physiologically, these acids fall into two groups: the volatile acid H_2CO_3 and all other nonvolatile or fixed acids. The difference between the two types of acids arises

because H_2CO_3 is in equilibrium with the volatile gas CO_2, which leaves the body by way of the lungs. The concentration of H_2CO_3 is therefore determined by the lungs and their respiratory capacity. The fixed acids (e.g., sulfuric, hydrochloric, phosphoric) are nonvolatile and are not eliminated by the lungs. Instead, they are buffered by body proteins or extracellular buffers, such as HCO_3^-, and then excreted by the kidney.

Carbon Dioxide and Bicarbonate Production

Body metabolism results in the production of approximately 15 000 mmol of CO_2 each day. Carbon dioxide is transported in the circulation in three forms:

- attached to hemoglobin
- dissolved CO_2 in the plasma
- as HCO_3^-

Collectively, dissolved CO_2 and $HCO3^-$ constitute approximately 77% of the CO_2 that is transported in the extracellular fluid; the remaining CO_2 travels attached to hemoglobin. Although CO_2 is not an acid, a small percentage of the gas combines with water in the bloodstream to form H_2CO_3.

The reaction that generates H_2CO_3 from CO_2 and water is catalyzed by an enzyme called carbonic anhydrase, which is present in large quantities in red blood cells, renal tubular cells, and other tissues in the body. The rate of the reaction between CO_2 and water is increased approximately 5000 times by the presence of carbonic anhydrase. Were it not for this enzyme, the reaction would occur too slowly to be of any significance.

Production of Noncarbonic Acids and Bases

The metabolism of dietary proteins and other substances results in the generation of noncarbonic acids and bases.

- Incomplete oxidation of glucose results in the formation of lactic acid.
- Incomplete oxidation of fats results in the production of ketoacids.
- Oxidation of the sulfur-containing amino acids (e.g., methionine, cysteine, cystine) results in the production of sulfuric acid.
- Oxidation of arginine and lysine produces hydrochloric acid.
- Oxidation of phosphorus-containing nucleic acids yields phosphoric acid.
- The major source of base is the metabolism of amino acids such as aspartate and glutamate, the metabolism of certain organic anions (e.g., citrate, lactate, acetate)
- A vegetarian diet, which contains large amounts of organic anions, results in the net production of base.

MECHANISMS OF ACID-BASE BALANCE

The pH is determined by the ratio of the bicarbonate (HCO_3^-) base to the volatile carbonic acid (H_2CO_3 / H^+ + HCO_3^-). At a normal pH of 7.4, the ratio is 20/1. The pH is regulated by extracellular (carbonic acid /bicarbonate) and intracellular (mainly proteins) systems that buffer changes in pH that would otherwise occur because of the metabolic production of volatile (CO_2) and nonvolatile (i.e., sulfuric and phosphoric) acids.

The respiratory system regulates the concentration of the volatile carbonic acid ($CO_2 + H_2O = H_2CO_3 = H^+ + HCO_3^-$) by changing the rate and depth of respiration. The kidneys regulate the plasma concentration of HCO_3^- by two processes:

reabsorption of the filtered HCO_3^- and generation of new HCO_3^- or the elimination of H^+ by tubular systems (phosphate and ammonia).

REGULATION OF pH

The pH of body fluids is regulated by intracellular and extracellular buffering systems that prevent large changes in the extracellular pH from occurring as well as through respiratory mechanisms that eliminate CO_2 and by renal mechanisms that conserve HCO_3^- ions and eliminate H^+ ions. The pH is further influenced by the electrolyte composition of the intracellular and extracellular compartments.

Intracellular and Extracellular Buffer Systems

A buffer system consists of a weak acid and the base salt of that acid or of a weak base and its acid salt. In the process of preventing large changes in pH, the system trades a strong acid for a weak acid or a strong base for a weak base.

The two major buffer systems that protect the pH of body fluids are proteins and the bicarbonate buffer system.

Bone also represents an important site for buffering of acids and bases. Although it is difficult to measure, it has been estimated that 40% of acute acid-base buffering occurs in bone. The role of bone buffers is even higher in chronic acid-base disorders. The consequence of bone buffering is the release of calcium and sodium from bone and increased renal excretion of calcium. In addition to causing demineralization of bone, it also predisposes to kidney stones.

Bicarbonate Buffer System.

The bicarbonate buffer system uses H_2CO_3 as its weak acid and a bicarbonate salt such as sodium bicarbonate $NaHCO_3$ as its weak base. It substitutes the weak H_2CO_3 for a strong acid such as hydrochloric acid ($HCl + NaHCO_3 = H_2CO_3 + NaCl$) or the weak bicarbonate base for a strong base such as sodium hydroxide ($NaOH + H_2CO_3 = NaHCO_3 + H_2O$). The HCO_3^-/CO_2 buffer system is a particularly efficient system because the buffer components can be readily added or removed from the body. Metabolism provides a supply of CO_2, which can replace any H_2CO_3 that

is lost when excess base is added, and CO_2 can be readily eliminated when excess acid is added. Likewise, the kidney can conserve or form new HCO_3^- when excess acid is added, and it can excrete HCO_3^- when excess base is added.

Protein Buffer Systems.

Proteins are the largest buffer system in the body. Proteins are amphoteric, meaning that they can function as acids or bases. They contain many ionizable groups that can release or bind H^+. The protein buffers are largely located in cells, and H^+ ions and CO_2 diffuse across cell membranes for buffering by intracellular proteins. Albumin and plasma globulins are the major protein buffers in the vascular compartment.

Plasma Potassium-Hydrogen Exchange.

Potassium ions (K+) and H^+ ions interact in important ways in the regulation of acid-base balance. Both ions are positively charged, and both ions move freely

between the intracellular and extracellular compartments. In situations of acidosis, excess H^+ ions move into the intracellular compartment for buffering. When this happens, another cation, in this case the K+ ion, must leave the cell and move into the extracellular fluid.

When extracellular potassium levels fall, K+ ions move out of the cell and are replaced by H^+ ions. Thus, alterations in extracellular potassium levels can affect acid-base balance, and changes in acid-base balance can influence extracellular potassium levels.

Respiratory Control Mechanisms

The second line of defense against acid-base disturbances is the control of CO_2 by the respiratory system. Excess CO_2 or excess H^+ ions in the blood mainly act directly on the respiratory center in the brain to control ventilation. It is the H^+ ion that stimulates the respiratory center, causing an increase or decrease in ventilation. The respiratory control of pH is rapid, occurring within minutes, and is maximal within 12 to 24 hours. Although the respiratory response is rapid, it does not completely return the pH to normal. It is only about 50% to 75% effective as a buffer system.

Renal Control Mechanisms

The kidneys regulate acid-base balance by excreting either an acidic or an alkaline urine. The renal mechanisms for regulating acid-base balance cannot adjust the pH within minutes, as respiratory mechanisms can, but they continue to function for days until the pH has returned to normal or near-normal range.

Hydrogen Ion Elimination and Bicarbonate Conservation.

The kidney regulates pH by excreting excess H^+ ions and reabsorbing or regenerating HCO_3^- ions. Bicarbonate is freely filtered in the glomerulus (approximately 4500 mEq/day) and reabsorbed in the proximal tubules. Because H^+ ions are not filtered in adequate amounts to maintain acid-base balance, they are

secreted from blood in the peritubular capillaries into the urine filtrate in the renal tubules.

Tubular Buffer Systems. There are two important intratubular buffer systems:

- the phosphate buffer system
- the ammonia buffer system

The phosphate buffer system uses HPO_4^{2-} and $H_2PO_4^-$ that are present in the tubular filtrate. The combination of H^+ with HPO_4^{2-} to form $H_2PO_4^-$ allows the kidneys to increase their secretion of H^+ ions.

Another important but more complex buffer system is the ammonia buffer system. The excretion of H^+ and generation of HCO_3^- by the ammonia buffer system occurs in a number of steps:

1. The metabolism of glutamate in the proximal tubule results in the formation of two NH_4 + and two HCO_3^- ions.

2. The two NH_4 + ions are secreted into the tubular fluid by a countertransport mechanism in exchange for a Na+ ion.

3. The two HCO_3^- ions move out of the tubular cell along with the reabsorbed Na+ ion to enter the peritubular capillaries system. Thus, for each molecule of glutamine metabolized in the proximal tubule, two NH_4 + ions are secreted into the tubular filtrate, and two HCO_3^- ions are reabsorbed into the blood.

4. A second buffering mechanism involves the recycling of NH_4+ by tubular cells in the medullary portion of the kidney. Here, NH_4+ is converted to NH_3 and secreted into the tubular lumen. In the collecting tubules, H+ ions that are secreted into the tubular lumen combine with NH_3 to form NH_4 + ions.

5. One of the most important features of the ammonia buffer system is that it is subject to physiologic control. Under normal conditions, the amount of H^+ ion eliminated by the ammonia buffer system is about 50% of the acid excreted and new HCO_3^- regenerated. However, with chronic acidosis, it can become the dominant mechanism for H^+ excretion and new HCO_3^- generation.

Hydrogen and Potassium Ions Compete for Elimination in the Urine.

Plasma K+ levels influence renal elimination of H^+ ions, and vice versa. When plasma K+ levels fall, there is movement of K+ ions from body cells into the extracellular fluid and a reciprocal movement of H^+ ions from the extracellular fluid into body cells. In the kidney, these movements lower the intracellular pH of tubular cells, causing an increase in H^+ ion secretion. An elevation in plasma K+ levels has the opposite effect.

Plasma K+ levels are similarly altered by acid-base balance. Acidosis tends to increase H+ ion elimination and decrease K+ ion elimination, with a resultant increase in plasma potassium levels. Alkalosis has the opposite effect.

Aldosterone also influences H^+ ion elimination by the kidney. It acts in the collecting duct to stimulate H+ ion secretion indirectly, while increasing Na+ ion reabsorption and K+ ion secretion. Hyperaldosteronism tends to lead to a decrease in

plasma K+ levels and increased pH and alkalosis because of increased H^+ ion secretion. Hypoaldosteronism has the opposite effect. It leads to increased K+ levels, decreased H^+ ion secretion, and acidosis.

The terms acidosis and alkalosis describe the clinical conditions that arise as a result of changes in dissolved CO_2 and HCO_3^- concentrations. Acidosis is what causes acidemia and alkalosis is what causes alkalemia. Hence, the term alkalosis has come to mean the opposite of acidosis.

METABOLIC VERSUS RESPIRATORY ACID-BASE DISORDERS

There are two types of acid-base disorders: metabolic and respiratory.

Metabolic disorders produce an alteration in bicarbonate concentration and result from the addition or loss of nonvolatile acid or alkali to or from the extracellular fluid. A reduction in pH due to a decrease in HCO_3^- is called metabolic acidosis, and an elevated pH due to increased HCO_3^- levels is called metabolic alkalosis.

Respiratory disorders involve an alteration in the pCO_2, reflecting an increase or decrease in alveolar ventilation. Respiratory acidosis is characterized by a decrease in pH, reflecting a decrease in ventilation and an increase in pCO_2. Respiratory alkalosis involves an increase in pH, resulting from an increase in alveolar ventilation and a decrease in pCO_2.

PRIMARY VERSUS COMPENSATORY MECHANISMS

Acidosis and alkalosis typically involve a primary or initiating event and a compensatory or adaptive state that results from homeostatic mechanisms that attempt to correct or prevent large changes in pH.

For example, a person may have a primary metabolic acidosis as a result of overproduction of ketoacids and respiratory alkalosis because of a compensatory increase in ventilation. Compensatory mechanisms adjust the pH toward a more normal level without correcting the underlying cause of the disorder. A mixed acid-base disorder is one in which there is both a primary and a compensatory change in acid-base balance.

The respiratory mechanisms, which compensate by increasing or decreasing ventilation, are rapid but seldom able to return the pH to normal because as the pH returns toward normal, the respiratory stimulus is lost. The kidneys compensate by conserving HCO_3^- or H^+ ions. It normally takes longer to recruit renal compensatory mechanisms than it does respiratory compensatory mechanisms. Renal mechanisms are more efficient, however, because they continue to operate until the pH has returned to a normal or near-normal value.

Compensation requires the use of mechanisms that are different from those that caused the primary disorder. In other words, the lungs cannot compensate for respiratory acidosis that is caused by lung disease, nor can the kidneys compensate for metabolic acidosis that occurs because of renal failure. The body can, however, use renal mechanisms to compensate for respiratory-induced changes in pH, and it can use respiratory mechanisms to compensate for metabolically induced changes in acid-base balance.

LABORATORY TESTS

Laboratory tests that are used in assessing acid-base balance include those for arterial blood gases and pH, CO_2 content and HCO_3^- levels (SB), buffer bases (BB) and base excess or deficit (BE).

Arterial blood (or capillary) gases are used because venous blood gases are highly variable, depending on metabolic demands of the various tissues that empty into the vein from where the sample is being drawn.

1. pH is useful in determining whether acidosis or alkalosis is present, and if it is compensated (pH within normal ranges) or decompensated (pH above or below normal ranges), but it provides little information about the cause of an acid-base disorder.

2. The pCO_2 of the arterial blood gases provides a means of assessing the respiratory component of acid-base balance. The dissolved CO_2 levels can be determined from arterial blood gas measurements using the pCO_2 (normal arterial pCO_2 is 35 to 45 mm Hg).

3. The plasma HCO_3^- (SB-standard bicarbonate) concentration is then determined from the total CO_2 content of the blood. The normal range of values for HCO_3^- concentration is 21 to 25 mEq/L (21 to 25 mmol/L).

4. The level of all the buffer systems of the blood-hemoglobin, protein, phosphate, and HCO_3^- (BB-buffer base) can be also measured. The normal range of values for BB is 46 to 52 mEq/L (46 to 52 mmol/L).

5. The base excess or deficit (BE) describes the amount of a fixed acid or base that must be added to a blood sample to achieve a pH of 7.4 (normal BE is ± 3.0 mEq/L or ±3.0 mmol/L).). 333Base excess indicates metabolic alkalosis, and base deficit indicates metabolic acidosis.

DISORDERS OF WATER AND ELECTROLYTE BALANCE

Fluids and electrolytes are present in body cells, in the tissue spaces between the cells, and in the blood that fills the vascular compartment. Body fluids transport gases, nutrients, and wastes, help generate the electrical activity needed to power body functions, take part in the transforming of food into energy and otherwise maintain the overall function of the body.

Composition and Compartmental Distribution of Body Fluids

Body fluids are distributed between the intracellular fluid (ICF) and extracellular fluid (ECF) compartments. The ICF compartment consists of fluid contained within all cells in the body. It is the larger of the two compartments, with approximately two thirds of the body water in healthy adults. The remaining one third of body water

is in the ECF compartment, which contains all the fluids outside the cells, including those in the interstitial or tissue spaces and blood vessels.

The ECF contains:

- large amounts of sodium and chloride
- moderate amounts of bicarbonate
- small quantities of potassium, magnesium, calcium, and phosphate

In contrast to the ECF, the ICF contains:

- almost no calcium
- small amounts of sodium, chloride, bicarbonate, and phosphate
- moderate amounts of magnesium
- large amounts of potassium

The cell membrane serves as the primary barrier to the movement of substances between the ECF and ICF compartments. Lipid-soluble substances such as gases (i.e.,

oxygen and carbon dioxide), which dissolve in the lipid bilayer of the cell membrane, pass directly through the membrane.

Many ions, such as sodium (Na^+) and potassium (K^+) rely on transport mechanisms such as the Na^+/K^+ pump that is located in the cell membrane for movement across the membrane (Na^+/K^+-ATPase membrane pump).

Water crosses the cell membrane by osmosis using special protein channels called aquaporins.

The movement of body fluids between the ICF and ECF compartments occurs at the cell membrane and depends on regulation of ECF water and sodium. Normally, equivalent changes in sodium and water are such that the volume and osmolality of ECF are maintained within a normal range. Because it is the concentration of sodium (in milligrams per liter) that controls ECF osmolality, changes in sodium are usually accompanied by proportionate changes in water volume. Protection of the circulatory volume can be viewed as the single most important characteristic of body fluid homeostasis.

Two mechanisms protect the ECF (and vascular fluid) volume: alterations in hemodynamic variables such as vasoconstriction and an increase in heart rate, and alterations in sodium and water balance.

Both mechanisms serve to maintain filling of the vascular compartment. Tachycardia, peripheral arterial vasoconstriction, and venoconstriction occur within

minutes of external fluid losses, whereas salt and water retention take hours to become effective.

REGULATION OF SODIUM BALANCE

Sodium is the most abundant cation in the body. Most of the body's sodium is in the ECF compartment (135 to 145 mEq/L), with only a small amount (10 to 14 mEq/L) located in the ICF compartment. The resting cell membrane is relatively impermeable to sodium. Sodium that enters the cell is transported out of the cell against an electrochemical gradient by the energy-dependent Na^+/K^+-ATPase membrane pump.

Sodium functions mainly in regulating extracellular and vascular volume. As the major cation in the ECF compartment, Na^+ and its attendant anions (Cl^+ and $HCO3^+$) account for approximately 90% to 95% of the osmotic activity in the ECF. Because sodium is part of the sodium bicarbonate molecule, it is important in regulating acid-base balance. As a current-carrying ion, Na^+ contributes to the function of the nervous system and other excitable tissue.

Gains and Losses

Sodium normally enters the body through the gastrointestinal tract and is eliminated by the kidneys or lost from the gastrointestinal tract or skin. Sodium intake normally is derived from dietary sources. Body needs for sodium usually can be met by as little as 500 mg/day. Most sodium losses occur through the kidney. The kidneys are extremely efficient in regulating sodium output, and when sodium intake is limited the kidneys are able to reabsorb almost all the sodium that has been filtered by the glomerulus. Conversely, urinary losses of sodium increase as intake increases.

Usually, less than 10% of sodium intake is lost through the gastrointestinal tract and skin. Sodium losses increase with conditions such as vomiting, diarrhea, fistula drainage, and gastrointestinal suction. Excessive amounts of sodium can also be lost through the skin. Sweat losses, which usually are negligible, can increase greatly during exercise, fever and periods of exposure to a hot environment. Loss of skin integrity, such as occurs in extensive burns, also leads to excessive skin losses of sodium.

Mechanisms of Regulation

The kidney is the main regulator of sodium. The kidney monitors arterial pressure and retains sodium when arterial pressure is decreased and eliminates it when arterial pressure is increased. The rate at which the kidney excretes or conserves sodium is coordinated by the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS). Another possible regulator of sodium excretion by the kidney is atrial natriuretic peptide (ANP), which is released from cells in the atria of the heart. ANP, which is released in response to atrial stretch and overfilling, increases sodium excretion by the kidney.

The Sympathetic Nervous System. The sympathetic nervous system responds to changes in arterial pressure and blood volume by adjusting (correcting) the glomerular filtration rate and thus the rate at which sodium is filtered from the blood. Sympathetic activity also regulates tubular reabsorption of sodium and renin release.

The Renin-Angiotensin-Aldosterone System. The RAAS exerts its action through angiotensin II and aldosterone. Renin is a small protein enzyme that is released by the kidney in response to changes in arterial pressure, the glomerular filtration rate, and the amount of sodium in the tubular fluid. Most of the renin that is released leaves the kidney and enters the bloodstream, where it interacts enzymatically to convert a circulating plasma protein called angiotensinogen to angiotensin I.

Angiotensin I is rapidly converted to angiotensin II by the angiotensin-converting enzyme in the small blood vessels of the lung. Angiotensin II acts directly on the renal tubules to increase sodium reabsorption. It also acts to constrict renal blood vessels, thereby decreasing the glomerular filtration rate and slowing renal blood flow so that less sodium is filtered and more is reabsorbed. Angiotensin II is also a powerful regulator of aldosterone, a hormone secreted by the adrenal cortex. Aldosterone acts at the level of the cortical collecting tubules of the kidneys to increase sodium reabsorption while increasing potassium elimination.

REGULATION OF WATER BALANCE

Total body water (TBW) varies with gender and weight. These differences can be explained by differences in body fat, which is essentially water free. Infants and young children have greater water content than adults. TBW constitutes approximately 75% to 80% of body weight in full-term infants, 60% in adult male, 50% in female.

Gains and Losses.

The main source of water gain is through oral intake and metabolism of nutrients. Water, including that obtained from liquids and solid foods, is absorbed from the gastrointestinal tract. Metabolic processes also generate a small amount of water.

Normally, the largest loss of water occurs through the kidneys, with lesser amounts being lost through the skin, lungs, and gastrointestinal tract. Water losses that occur through the skin and lungs are referred to as insensible water losses because they occur without a person's awareness. The gains and losses of body water are summarized here:

Gains		Losses	
Oral intake		Urine	1500 mL
As water	1000 mL	Insensible losses	
In food	1300 mL	Lungs	300 mL
Water of oxidation	200 mL	Skin	500 mL
		Feces	200 mL
Total	2500 mL	Total	2500 mL

Mechanisms of Regulation

There are two main physiologic mechanisms that assist in regulating body water:

- thirst
- antidiuretic hormone (ADH)

Thirst is primarily a regulator of water intake and ADH a regulator of water output. Both mechanisms respond to changes in extracellular osmolality and volume. Like appetite and eating, thirst and drinking behavior are two separate entities. Thirst is a conscious sensation of the need to obtain and drink fluids high in water content. Most people drink without being thirsty, and water is consumed before it is needed. As a result, thirst is basically an emergency response. Thirst is controlled by the thirst center in the hypothalamus. There are two stimuli for true thirst based on water need:

1) cellular dehydration caused by an increase in extracellular osmolality,

2) a decrease in blood volume, which may or may not be associated with a decrease in serum osmolality.

Sensory neurons, called osmoreceptors, which are located in or near the thirst center in the hypothalamus, respond to changes in extracellular osmolality by swelling or shrinking. Thirst normally develops when there is as little as a 1% to 2% change in serum osmolality.

Stretch receptors in the vascular system that are sensitive to changes in arterial blood pressure (high-pressure baroreceptors located in the carotid sinus and aorta) and central blood volume (low-pressure baroreceptors located in the left atrium and major thoracic veins) also aid in the regulation of thirst.

A third important stimulus for thirst is angiotensin II, which becomes increased in response to low blood volume and low blood pressure. The renin-angiotensin mechanism contributes to nonosmotic thirst. Elevated levels of angiotensin II may lead to thirst in conditions, such as chronic renal failure and congestive heart failure, in which renin levels may be elevated. Thirst and elevated renin levels are also found in persons with primary hyperaldosteronism and in those with secondary

hyperaldosteronism accompanying anorexia nervosa, hemorrhage, and sodium depletion.

Antidiuretic Hormone. The reabsorption of water by the kidneys is regulated by ADH, also known as vasopressin. ADH is synthesized by cells in the supraoptic and paraventricular nuclei of the hypothalamus. ADH from neurons in the supraoptic and paraventricular nuclei is transported along a neural pathway (i.e., hypothalamohypophysial tract) to the neurohypophysis (i.e., posterior pituitary) and then stored for future release. ADH exerts its effects through two types of vasopressin (V) receptors - V1 and V2 receptors. V1 receptors, which are located in vascular smooth muscle, cause vasoconstriction-hence the name vasopressin. Although ADH can increase blood pressure through V1 receptors, this response occurs only when ADH levels are very high. The V2 receptors, which are located on the tubular cells of the cortical collecting duct, control water reabsorption by the kidney. Binding of ADH to the V2 receptors increases water reabsorption by increasing the permeability of the collecting duct to water (i.e., the antidiuretic effect).

As with thirst, ADH levels are controlled by extracellular volume and osmolality. Osmoreceptors in the hypothalamus sense changes in extracellular osmolality and stimulate the production and release of ADH. Likewise, stretch receptors that are sensitive to changes in blood pressure and central blood volume aid in the regulation of ADH release.

The abnormal synthesis and release of ADH occurs in a number of stress situations. Severe pain, nausea, trauma, surgery, certain anesthetic agents, and some analgesic drugs increase ADH levels. Nausea is a potent stimulus of ADH secretion. Among the drugs that affect ADH are nicotine, which stimulates its release, and alcohol, which inhibits it.

COMPARTMENTAL DISTRIBUTION OF BODY FLUIDS

Body water is distributed between the Intracellular Fluid (ICF) and Extracellular Fluid (ECF) compartments. In the adult, the fluid in the ICF compartment constitutes approximately 40% of body weight.

The fluid in the ECF compartment is further divided into two major subdivisions: the plasma compartment, which constitutes approximately 4% of body weight, and the interstitial fluid compartment, which constitutes approximately 15% of body weight.

A third, usually minor, subdivision of the ECF compartment is the transcellular compartment, which is defined as being separated by a layer of epithelium. It includes the cerebrospinal fluid and fluid contained in the various body spaces, such as the peritoneal, pleural, and pericardial cavities; the joint spaces; and the gastrointestinal tract. Normally, only approximately 1% of ECF is in the transcellular space. This amount can increase considerably in conditions such as ascites, in which large amounts of fluid are sequestered in the peritoneal cavity.

Intracellular Fluid Volume.

The ICF volume is regulated by proteins and organic compounds in the ICF and by solutes that move between the ECF and ICF. The membrane in most cells is freely

permeable to water; therefore, water moves between the ECF and ICF fluid as a result of osmosis. In contrast, osmotically active proteins and other organic compounds cannot pass through the membrane.

Water entry into the cell is regulated by these osmotically active substances as well as by solutes such as sodium and potassium that pass through the cell membrane. Many of the intracellular proteins are negatively charged and attract positively charged ions such as the K^+ ion, accounting for its higher concentration in the ICF.

The Na^+ ion, which has a greater concentration in the ECF, tends to enter the cell by diffusion. The Na^+ ion is osmotically active, and its entry would, if left unchecked, pull water into the cell until it ruptured. The reason this does not occur is because the Na^+/K^+-ATPase membrane pump continuously removes three Na^+ ions from the cell for every two K^+ ions that are moved back into the cell. Situations that impair the function of the Na^+/K^+-ATPase pump, such as hypoxia, cause cells to swell because of an accumulation of Na^+ ions.

Other ions, such as $Ca^{2+}$and H^+, are exchanged by similar transport systems. Intracellular volume is also affected by the concentration of osmotically active substances in the ECF that cannot cross the cell membrane. In diabetes mellitus, for example, glucose cannot enter the cell, and its increased concentration in the ECF pulls water out of the

Extracellular Fluid Volume.

The ECF volume is divided between the vascular and interstitial fluid compartments. The vascular compartment contains blood, which is essential to the transport of substances such as electrolytes, gases, nutrients, and waste products throughout the body. Interstitial fluid acts as a transport vehicle for gases, nutrients, wastes, and other materials that move between the vascular compartment and body cells. Interstitial fluid also provides a reservoir from which vascular volume can be maintained during periods of haemorrhage or loss of vascular volume.

A tissue gel, which is a spongelike material composed of large quantities of mucopolysaccharides, fills the tissue spaces and aids in even distribution of interstitial fluid. Normally, most of the fluid in the interstitium is in gel form. The tissue gel is supported by collagen fibers that hold the gel in place. The tissue gel, which has a firmer consistency than water, opposes the outflow of water from the capillaries and prevents the accumulation of free water in the interstitial spaces.

RELATION OF PLASMA SODIUM CONCENTRATION TO OSMOLALITY.

The osmolality of the plasma P_{osm} is equal to the sum of the osmolalities of the individual solutes in the plasma – Na, glucose, urea. The normal values are:

$$P_{osm} = 2\times\text{plasma } Na^+ + \text{glucose (mosmol\kg)} + \text{BUN (Mosmol\kg)}$$

Plasma Na^+=135-145 meq/l, Glucose=60-100 mg%, BUN=10-20 mg%

Mosmol\kg = mg%×10 / mol. weight

P_{osm} = 2×plasma Na^+ + glucose (mg%)/18 + BUN/2.8

Since urea is an ineffective osmole,

Effective P_{osm}=2×plasma Na^+ + glucose (mg%)/18

P_{osm}=275-290 mosmol\kg

Effective P_{osm}=270-285 mosmol\kg

Example 1: A patient has the following laboratory data:
Plasma Na^+=125 meq/l, Glucose=108 mg%, BUN=140 mg%.
1. Calculate the plasma osmolality.
2. Would this patient have the symptoms of hyperosmolality?

Sodium deficit

Na deficit = TBW(volume of distribution of plasma Na) × Na deficit per liter
TBW – total body water
Na deficit per liter = 120-current Na

Example 2: 60 kg woman, plasma Na concentration – 108 meq/l
1. How much Na will she need?
2. Since 3% saline contains 513 meq/l of Na, how many ml of this solution will provide the required Na?

Water deficit

Total body osmoles = TBW× P_{osm} (or plasma Na),

Current body water CBW× plasma Na = Normal body water NBW× 140 (plasma Na)
NBW = CBW× plasma Na/140
Water deficit = NBW-CBW
Water deficit = [CBW× plasma Na/140] –CBW
Water deficit = CBW × [plasma Na/140-1]

Example 3: Woman with the plasma Na 183 meq/l, body weight 50 kg will have…..water deficit?

CAPILLARY-INTERSTITIAL FLUID EXCHANGE

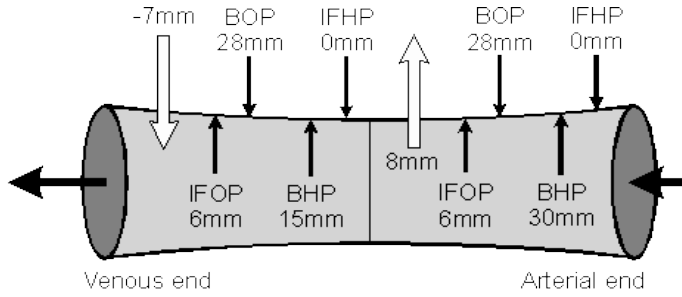
The transfer of water between the vascular and interstitial compartments occurs at the capillary level and is governed by the Starling forces (Fig. 6). Four forces control the movement of water between the capillary and interstitial spaces:

1) the capillary filtration (hydrostatic) pressure, which pushes water out of the capillary into the interstitial spaces;

2) the capillary colloidal osmotic pressure, which pulls water back into the capillary;

3) the interstitial fluid pressure, which opposes the movement of water out of the capillary;

4) the tissue colloidal osmotic pressure, which pulls water out of the capillary into the interstitial spaces.



BHP = Blood hydrostatic pressure
IFHP = Interstitial fluid hydrostatic pressure
BOP = Blood osmotic pressure
IFOP = Interstitial fluid osmotic pressure

Figure 6 .Starling forces

Normally, the combination of these four forces is such that only a small excess of fluid remains in the interstitial compartment. This excess fluid is removed from the interstitium by the lymphatic system and returned to the systemic circulation. Capillary filtration refers to the movement of water through capillary pores because of a mechanical rather than an osmotic force.

The capillary filtration pressure, sometimes called the capillary hydrostatic pressure, is the pressure pushing water out of the capillary into the interstitial spaces. It reflects the arterial and venous pressures, the precapillary (arterioles) and postcapillary (venules) resistances, and the force of gravity.

- A rise in arterial or venous pressure increases capillary pressure.
- A decrease in arterial resistance or increase in venous resistance increases capillary pressure.
- An increase in arterial resistance or decrease in venous resistance decreases capillary pressure.

The force of gravity increases capillary pressure in the dependent parts of the body. In a person who is standing absolutely still, the weight of blood in the vascular column causes an increase of 1 mm Hg in pressure for every 13.6 mm of distance from the heart. This pressure results from the weight of water. This pressure is then transmitted to the capillaries.

The capillary colloidal osmotic pressure is the osmotic pressure generated by the plasma proteins that are too large to pass through the pores of the capillary wall. The term colloidal osmotic pressure differentiates this type of osmotic pressure from the osmotic pressure that develops at the cell membrane from the presence of electrolytes and nonelectrolytes. Because plasma proteins do not normally penetrate the capillary pores and because their concentration is greater in the plasma than in the interstitial fluids, it is capillary colloidal osmotic pressure that pulls fluids back into the capillary.

The interstitial fluid pressure and the tissue colloidal osmotic pressure contribute to movement of water into and out of the interstitial spaces. The interstitial fluid pressure opposes the outward movement of water from the capillary into the interstitial spaces. The tissue colloidal osmotic pressure pulls water out of the capillary into the tissue spaces. It reflects the small amount of plasma proteins that normally escape from the capillary to enter the interstitial spaces.

EDEMA

Edema can be defined as palpable swelling produced by expansion of the interstitial fluid volume. Edema does not become clinically evident until the interstitial volume has been increased by 2.5 to 3 L. The physiologic mechanisms that contribute to edema formation include factors that:

- increase the capillary filtration (hydrostatic) pressure
- decrease the capillary colloidal osmotic pressure
- increase capillary permeability
- produce obstruction to lymph flow

Increased Capillary Filtration Pressure. As the capillary filtration pressure rises, the movement of vascular fluid into the interstitial spaces increases. Among the factors that increase capillary pressure are:

- increased arterial pressure or decreased resistance to flow through the precapillary sphincters (precapillary vasodilation),
- an increase in venous pressure or increased resistance to outflow at the postcapillary sphincter (venous obstruction),
- capillary distention due to increased vascular volume.

Edema can be either localized or generalized. The localized edema that occurs with allergic or inflammatory conditions results from the release of histamine and other inflammatory mediators that cause dilation of the precapillary sphincters and arterioles (precapillary vasodilation), that supply the injured tissue and venous obstruction due to thrombosis, increased blood viscosity and mechanical compression. Thrombophlebitis obstructs venous flow, producing an elevation of venous pressure and edema of the affected part, usually one of the lower extremities.

Generalized edema is common in conditions such as congestive heart failure that produce fluid retention and venous congestion. In right-sided heart failure, blood dams up throughout the entire venous system, causing organ congestion and edema of the dependent extremities. Decreased sodium and water excretion by the kidneys leads to

an increase in extracellular volume, with an increase in capillary volume and pressure and subsequent movement of fluid into the tissue spaces.

Because of the effects of gravity, edema resulting from increased capillary pressure commonly causes fluid to accumulate in the dependent parts of the body, a condition referred to as dependent edema. For example, edema of the ankles and feet becomes more pronounced during prolonged periods of standing.

Decreased Capillary Colloidal Osmotic Pressure. Plasma proteins exert the osmotic force needed to pull fluid back into the capillary from the tissue spaces. Because of its lower molecular weight, 1 g of albumin has approximately twice as many osmotically active molecules as 1 g of globulin and almost six times as many osmotically active molecules as 1 g of fibrinogen. Also, the concentration of albumin (approximately 4.5 g/dL) is greater than that of the globulins (2.5 g/dL) and fibrinogen (0.3 mg/dL).

Edema due to decreased capillary colloidal osmotic pressure usually is the result of inadequate production or abnormal loss of plasma proteins, mainly albumin. The plasma proteins are synthesized in the liver. In persons with severe liver failure, impaired synthesis of albumin results in a decrease in colloidal osmotic pressure. In starvation and malnutrition, edema develops because there is a lack of amino acids needed in plasma protein synthesis.

The most common site of plasma protein loss is the kidney. In kidney diseases such as nephrotic syndrome, the glomerular capillaries become permeable to the plasma proteins, particularly albumin, which is the smallest of the proteins. When this happens, large amounts of albumin are filtered out of the blood and lost in the urine. An excessive loss of plasma proteins also occurs when large areas of skin are injured or destroyed. Edema is a common problem during the early stages of a burn, resulting from capillary injury and loss of plasma proteins.

Because the plasma proteins are evenly distributed throughout the body and are not affected by the force of gravity, edema due to a decrease in capillary colloidal osmotic pressure tends to affect tissues in nondependent as well as dependent parts of the body. There is swelling of the face as well as the legs and feet.

Increased Capillary Permeability. When the capillary pores become enlarged or the integrity of the capillary wall is damaged, capillary permeability is increased. When this happens, plasma proteins and other osmotically active particles leak into the interstitial spaces, increasing the tissue colloidal osmotic pressure and thereby contributing to the accumulation of interstitial fluid. Among the conditions that increase capillary permeability are burn injury, capillary congestion, inflammation, and immune responses.

Obstruction of Lymph Flow. Osmotically active plasma proteins and other large particles that cannot be reabsorbed through the pores in the capillary membrane rely on the lymphatic system for movement back into the circulatory system. Edema due to impaired lymph flow is commonly referred to as lymphedema. Malignant involvement

of lymph structures and removal of lymph nodes at the time of cancer surgery are common causes of lymphedema. Another cause of lymphedema is infection involving the lymphatic channels and lymph nodes.

Manifestations. The effects of edema are determined largely by its location. Edema of the brain, larynx, or lungs is an acute, life-threatening condition. Although not life threatening, edema may interfere with movement, limiting joint motion. Swelling of the ankles and feet often is insidious in onset and may or may not be associated with disease. At the tissue level, edema increases the distance for diffusion of oxygen, nutrients, and wastes. Edematous tissues usually are more susceptible to injury and development of ischemic tissue damage, including pressure ulcers. Edema can also compress blood vessels.

Assessment and Treatment. Methods for assessing edema include daily weight, visual assessment, measurement of the affected part, and application of finger pressure to assess for pitting edema. Daily weight performed at the same time each day with the same amount of clothing provides a useful index of water gain due to edema. Visual inspection and measurement of the circumference of an extremity can also be used to assess the degree of swelling. This is particularly useful when swelling is due to thrombophlebitis. Finger pressure can be used to assess the degree of pitting edema. If an indentation remains after the finger has been removed, pitting edema is identified.

Diuretic therapy commonly is used to treat edema. Edema of the lower extremities may respond to simple measures such as elevating the feet. Serum albumin levels can be measured, as can the colloidal osmotic pressure of the plasma (normally approximately 25.4 mm Hg). Albumin can be administered intravenously to raise the plasma colloidal osmotic pressure when edema is caused by hypoalbuminemia.

Third-Space Accumulation. Third spacing represents the loss of ECF into the transcellular space. The serous cavities are located in strategic body areas where there is continual movement of body structures-the pericardial sac, the peritoneal cavity, and the pleural cavity. The exchange of ECF between the capillaries, the interstitial spaces, and the transcellular space of the serous cavity uses the same mechanisms as capillaries elsewhere in the body. The prefix hydro- may be used to indicate the presence of excessive fluid, as in hydrothorax, which means excessive fluid in the pleural cavity. The accumulation of fluid in the peritoneal cavity is called ascites.

INFLAMMATION

Lesson 1

Inflammation is the defending response of the living body to the local tissues damage. Inflammation occurs in a defined order: first eliminating foreign pathogens, and then remodelling tissue, thereby establishing homeostasis.

- First, the activation of resident cells (mast cells, resident macrophages and dendritic cells) and rapid entry of granulocytes in response to injury.
- Second, further recruitment of macrophages.
- Third, infiltration of effector immune cells (lymphocytes) to enable specific immune responses.

• Fourth, the recruitment and activation of mesenchymal cells such as endothelial cells and fibroblasts to form new blood vessels and a collagenous matrix.

• Fifth, tissue remodelling.

In its initial stages, inflammation is an aggressive state that can destroy both exogenous pathogens and host tissues. This is followed by a switch to a state that promotes cell survival and tissue regeneration.

Sequence of Acute inflammation

There are 3 main stages in acute inflammation:

- Alteration stage (tissue damage).
- Vascular stage (changes in blood flow, vascular permeability with the following involvement of cellular and humoral factors into the damage area).
- Proliferation (healing and tissue repair stage).

ALTERATION STAGE

Alteration is a process reflecting the tissue damage upon the action of various provoking agents called phlogogenic factors. This stage is accomplished with physical and chemical changes in inflammatory site.

1) Increased osmolarity due to a) tissue destruction → accumulation of the intracellular ions (K+) and protons (H+); b) activation of tissue metabolism (activation of proteolysis glucolysis, lypolysis) → increased synthesis and release of organic acids (tricarboxylic acid released from Krebs cycle), fatty acids, polypeptides and amino acids.

2) Acidosis.

3) Increased oncotic pressure due to a) activation of lyzosomal enzymes with proteolityc activities → the release of enzymes into an extracellular space → tissue destruction and proteolysis→ accumulation of polypeptides and amino acids; b) increased vascular permeability → accumulation of plasma proteins in the tissues.

There are 2 principle varieties of the alteration: primary and secondary.

Primary alteration occurs as a consequence of the direct action of phlogogenic factors with the following damage of tissue structure.

Secondary alteration is related to the release of the enzymes, acids, free radicals (oxidative stress) and inflammatory mediators into the extracellular space with the following tissue destruction. Thereby, secondary alteration can increase the area of tissue damage even after the action and phlogogenic agent is finished.

VASCULAR STAGE

Essentially, there are five main steps in the vascular stage:

- Transient period of vasoconstriction.

- Increased blood flow and vascular permeability (active or arterial hyperemia).
- Decreased blood flow accomplished with the emigration of cellular and humoral elements out of blood vessels and into the damaged area (passive or venous hyperemia).
- Pendulum-like blood flow.
- Stop of the blood flow (stasis).

1) Vasoconstriction. After the initial injury, there is a transient period of vasoconstriction lasting from a few seconds to a few minutes. The vasoconstriction is a consequence of the release of noradrenaline, adrenaline, dopamine etc. The duration of this phase depends on the severity of the injury; the intensity of the following release of mediators with antagonistic properties. Since the mediators with vasoconstictive activities are degraded by enzymes becoming active only when tissue acidosis occurs, and the intensity of pH decrease correlates with the severity of the injury, becomes clear that during significant injuries the vasoconstriction period may be relatively short or even not occur.

2) Arterial (active) hyperemia. After the vasoconstriction vasodilatation occurs, predominantly in arterioles. Capillary flow is also increased through the relaxation of pre-capillary sphincters. With decreased resistance, increased blood flow occurs: the clinical swelling and redness result. After blood flow has initially increased (a variable period, usually minutes), there comes a period of increased vascular permeability and the walls of the microcirculation become more permeable to protein-rich fluid, resulting in exudation of plasma proteins and tissue edema. Thereby, the normal balance of capillary oncotic and hydrostatic pressure is disrupted. The loss of this fluid results in concentration of the intravascular cellular elements and increased blood viscosity. The slowing of flow facilitates approximation of leukocytes with the walls of the vessels, ready for the next stages.

For mild injury, released inflammatory mediators such as histamine and bradykinin act on venules to cause endothelial cell contraction, thus opening gaps between cells. More severe injury may result in a simple mechanical breach of the endothelial wall.

Other mediators involved with increasing vascular permeability include:

- complement components C3a and C5a,
- leukotrienes C4, D4 and E4 (also named as a slow-reactive substance of anaphylaxy SRS-A),
- prostaglandins PGE1 and PGE2,
- platelet activating factor (PAF).

3) Venous (passive) hyperemia. The major difference of this type of hyperemia from the previous vascular stage consists in the decrease of the blood outflow. There are 2 groups of factors causing this phenomenon: extravascular and intravascular.

- The extravascular factors include: a) the exudates, which are squeezing predominantly the venules; b) the destruction of the vessel cytoskeleton as a result of acidosis.
- The intravascular factors include: a) the increase of the blood viscosity as a result of exudation; b) activation of proteins with the coagulative activities (tissue factor, factor XII, protrombine); c) leukocytes rolling (margination) and adhesion to the endothelial cells, RBC aggregation.

4) Pendulum-like blood flow. Pendulum-like blood flow is a consequence of the significant decrease of the blood outflow. In systoles the blood moves by its usual way (arterioles→capillaries→venules). In diastoles the blood moves retrogradually (venules →capillaries→ arterioles). As a result of the transient increase of hydrostatic pressure in venules (in systoles) and irritation of nervous terminations, the so-called "beating pain" occurs.

5) Stop of the capillary flow (stasis). The following changes occur consistently in this stage: a) erythrocytes draw together, this phenomenon is named "aggregation". It is reversible phenomenon. If the changes on blood flow will be normalized, the erythrocytes can be dispersed and restore the function; b) erythrocytes stick each other. This phenomenon is named as "agglutination". It is irreversible phenomenon occurring in the prolonged period of the stasis. Even the blood flow will be normalized the previously agglutinated erythrocytes will be not able to restore their function; c) "sludge phenomenon" is characterized by the fusion of blood cells and the absence of borders between the plasma and cells in the blood.

Factors involved in vascular permeability in acute inflammation.

The ultrastructural basis of increased vascular permeability was originally determined using an experimental model in which histamine, one of the chemical mediators of increased vascular permeability, was injected under the skin. This caused transient leakage of plasma proteins into the extravascular space. Electron microscopic examination of venules and small veins during this period showed that gaps of 0.1-0.4 μm in diameter had appeared between endothelial cells. These gaps allowed the leakage of injected particles, such as carbon, into the tissues.

The endothelial cells are not damaged during this process. They contain contractile proteins such as actin, which, when stimulated by the chemical mediators of acute inflammation, cause contraction of the endothelial cells, pulling open the transient pores. The leakage induced by chemical mediators, such as histamine, is confined to venules and small veins. Although fluid is lost by ultra filtration from capillaries, there is no evidence that they too become more permeable in acute inflammation.

In addition to the transient vascular leakage caused by inflammatory mediators, certain other stimuli, e.g. heat, cold, ultraviolet light and X-rays, bacterial toxins and corrosive chemicals, cause delayed prolonged leakage. In these circumstances, there is direct injury to endothelial cells in several types of vessels within the damaged area.

The relative importance of chemical mediators and of direct vascular injury in causing increased vascular permeability varies according to the type of tissue. For example, vessels in the central nervous system are relatively insensitive to the chemical mediators, while those in the skin, conjunctiva and bronchial mucosa are exquisitely sensitive to agents such as histamine.

Summing above disscussed, there are two mechanisms for increased permeability of small vessels following tissue damage:

- toxins and physical agents may cause necrosis of vascular endothelium, leading to abnormal leakage (non-mediated vascular leakage),
- chemical mediators of acute inflammation may cause retraction of endothelial cells, leaving intercellular gaps (mediated vascular leakage).

Three patterns of increased leakage of fluid from vessels, which occur at different times following injury:

- An immediate response that is transient, lasts for 30-60 minutes, and is mediated by histamine acting on endothelium.
- An immediate response that is prolonged for over 24 hours and is seen if there has been direct necrosis of endothelium, e.g. in a bum or by a chemical toxin.
- A delayed response that starts 2-3 hours after injury and lasts for up to 8 hours. This is mediated by factors synthesized by local cells, e.g. prostaglandins, leukotriens, bradykinin; factors derived from complement; and factors released from dead neutrophils (enzymes) in the exudates.

In disease it is likely that all three responses are activated, with an immediate prolonged response close to the center of damage, and mediated responses at the interface between the damaged and healthy tissues.

Mechanisms and of exudation

In acute inflammation the following forces make much more fluid to leave the vessels than return to them:

- capillary hydrostatic pressure is increased, as a result of increased inflow and decreased outflow of blood;
- vascular permeability is increased under the influence of mediators;
- colloidal-osmotic pressure in the tissue is increased, as there is escape of plasma proteins into the extra vascular space, accumulation of the intracellular ions, activation of lyzosomal enzymes with proteolityc activities.

The net escape of protein-rich fluid is called exudation; hence, the fluid is called the fluid exudate.

Exudates and transudates. An exudate describes an inflammatory fluid emanating from the intravascular space due to changes in the permeability of the microcirculation. The increased vascular permeability means that large molecules, such as proteins, can escape from vessels. Hence, the exudate fluid has a high protein content of over 3%

and has a specific gravity greater than 1.020. There is a considerable turnover of the inflammatory exudate it is constantly drained away by local Iymphatic channels to be replaced by new exudate.

The proteins present include:

- immunoglobulins, which may be important in the destruction of antigens and invading microorganisms;
- acute phase proteins serving as inflammatory mediators, opsonizing foreign organisms and particles, neutralizing free radicals and proteolytic enzymes, transporting damaged components;
- coagulation factors, including fibrinogen, which esult in fibrin deposition on contact with the extra vascular tissues, hence, acutely inflamed organ surfaces are commonly covered by fibrin.

Exudates should be contrasted with a transudate. A transudate is a fluid emanating from the intravascular compartment due to an imbalance of the hydrostatic forces across the walls of the microcirculation. Clinically, it has low protein content and a specific gravity of less than 1.012. Transudate is defined as having less than 3 g of protein per 100 ml of fluid.

Components of the acute inflammation

Acute inflammation brings a range of cell types and inflammatory mediators into the area of damage. Cellular elements:

- Polymorphonuclear granulocytes (neutrophils) – appear early and have key role in removing foreign material and bacteria, necrotic cells, release inflammatory mediators. They have a life-span of only 13 days and must be constantly replaced; most die locally, but some leave the site via the lymphatics.
- Monocytes-macrophages – blood monocytes arrive at the site and, on leaving the blood vessels, transform into macrophages, becoming more metabolically active, and phagocytic. Appear later in the response, are usually responsible for clearing away tissue debris and damaged cells, release inflammatory mediators (cytokines-monokines) with a significant role in wound healing, act as antigen presenting cells.

Both neutrophils and macrophages may discharge their lysosomal enzymes into the extracellular fluid by exocytosis, or the entire cell contents may be released when the cells die. Release of these enzymes assists in the digestion of the inflammatory exudates.

- Eosinophils – active role in certain parasitic infections and hypersensitivity.
- Mast cells – release mediators acting locally e.g. preformed mediators histamine, heparin, and newly formed lipid mediators (eicosanoids).
- Endothelial cells – can produce mediators, on activation express adhesion molecules and migrate during the process of angiogenesis.

- Platelets – induce blood clotting, release mediators, with a significant role in wound healing.

The type of leukocytes in the inflammatory tissues in transit depends on the stage of inflammation and the stimulus:

- usually, polymorphonuclear neutrophils predominate in the first 24 hours disappearing by 36 hours;
- monocytes become more frequent after 24 hours and may stay at the site of chronic inflammation for long periods;
- viral infections may promote lymphocyte transit initially;
- some hypersensitivity reactions promote initial movement of eosinophils.

Chemical inflammatory mediators: tissue derived – histamine, serotonin, leukotrienes, prostaglandins, cytokines; plasma derived – kinin, complement, clotting, fibrinolytic systems.

Movement of cellular elements to the inflammatory site

The most important cells in acute inflammation are leukocytes. These need to pass from the blood to the site of activity across the endothelium; they do so in overlapping stages (Fig. 7):

- margination
- adhesion
- passage across vessel wall
- chemotaxis

Margination – the way in which the polymorphonuclear leukocytes come to lie adjacent to the capillary walls. In the normal circulation, cells are confined to the central (axial) stream in blood vessels, and do not flow in the peripheral (plasmatic) zone near to the endothelium. However, loss of intra vascular fluid and increase in plasma viscosity with slowing of flow at the site of acute inflammation allows leukocytes to flow in this plasmatic zone. The adhesion of leukocytes to endothelium causes them to aggregate along the vessel walls in a process termed margination.

Adhesion – the leukocyte alongside the endothelial cell adheres to it by means of a complex interaction between complementary adhesion molecules. There are two phases of adherence: the selectin-dependent phase and the integrin-dependent phase.

Within minutes, released histamine and thrombin stimulate the appearance of a molecule, P-selectin of the endothelial cell surface. Later, interleukin-1 and tumour necrosis factor promote the production of another endothelial molecule, E-selectin. The adherence of phagocytes to vascular endothelium is a dynamic process which is initiated by a phenomenon called "rolling." Increased rolling has been referred to as "margination" by histologists.

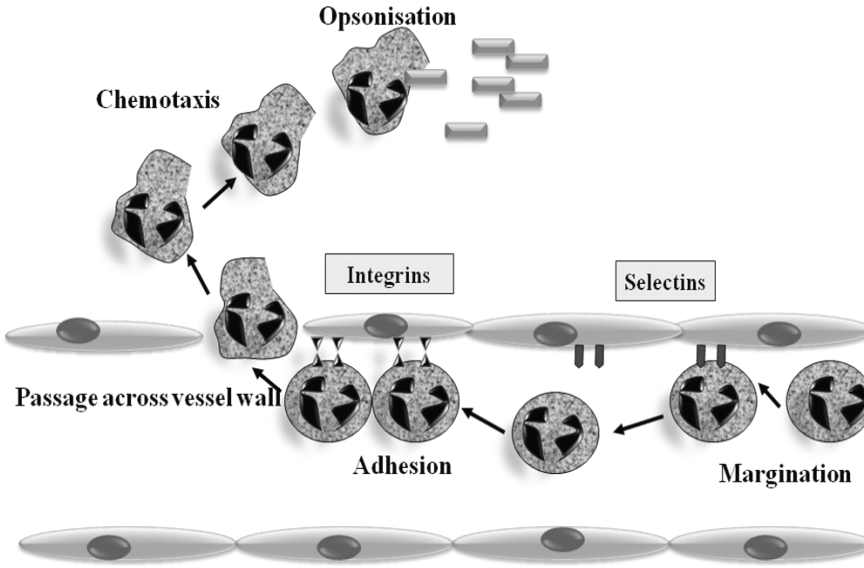


Figure 7. Movement of neutrophils to the inflammatory site

Notice how the endothelium, not the phagocyte, initially responds to the inflammatory signal. The binding specificities of both P-selectin and E-selectin are for carbohydrates of leukocyte surface glycoproteins. Selectins retard the movement of the marginating leukocyte but do not halt it completely. This can only occur with the expression of a second-wave of binding proteins. On the leukocyte, these are termed beta2 integrins: LFA-1 (leukocyte function-associated molecule). Their complementary molecules on the endothelium are intercellular adhesion molecules - ICAMs and vascular adhesion molecules VCAMs. Disorder within the adhesion molecule structure can lead to a failure of leukocytes to reach the region of inflammation - leukocyte adhesion deficiency.

Passage across vessel wall – once adhered, the leukocyte moves a section of itself - a pseudopodium - through an intercellular junction. This process depends on the relatively high concentration of ICAM-1 and VCAM adhesion molecules at the site. Leukocytes migrate by active amoeboid movement through the walls of venules and small veins, but do not commonly exit from capillaries. Electron microscopy shows that neutrophil, eosinophil granulocytes and also macrophages can insert pseudopodia between endothelial cells, migrate through the gap so created between the endothelial cells, and then on through the basal lamina into the vessel wall. The defect appears to be self-sealing, and this process does not damage the endothelial cells.

Red cells may also escape from vessels, but in this case the process is passive and depends on hydrostatic pressure forcing the red cells out. The process is called

diapedesis, and the presence of large numbers of red cells in the extravascular space implies severe vascular injury, such as a tear in the vessel wall.

Chemotaxis – the movement of leukocytes from the vessel lumen into the damaged area is mediated by substances known as chemotactic factors, which diffuse from the area of tissue damage. Therefore, chemotaxis is the movement of a cell or organism towards a stimulus, usually along the gradient of a diffusible chemical signal. It must be distinguished from chemokinesis, where the movement is not directed. A classical example is the movement of the neutrophils towards an area of acute inflammation. The main neutrophil chemotactic factors are:

- bacterial components e.g. simple peptides;
- components of complement system C3a, C5a;
- endogenous arachidonic acid metabolite leukotriene B4;
- cytokines (chemokines).

These factors bind to receptors on the surface of the cell and activate secondary messenger systems, (e.g. increased cytosolic calcium), with resulting to the assembly of internal cytoskeletal specializations involved in motility and thereby producing movement in a given direction. Contraction of cytoplasmic microtubules and gel/sol changes in cytoplasmic fluidity bring about amoeboid movement. These active mechanisms are dependent upon calcium ions and are controlled by intracellular concentrations of cyclic nucleotides. The movement shows a directional response (chemotaxis) to various chemicals.

Phagocytosis

Phagocytosis – the process whereby cells (neutrophils and macrophages) ingest solid particles. Adhesion of the particle to be engulfed to the cell surface is the first step in phagocytosis. This is facilitated by opsonization. The phagocyte then ingests the attached particle by sending out pseudopodia around it. These meet and fuse so that the particle lies in a phagocytic vacuole (also called a phagosome) bounded by cell membrane. Lysosomes, membrane-bound packets containing the digestic compounds described below, then fuse with phagosomes to form phagolysosomes. It is within these that intracellular killing of microorganisms occurs.

Adhesion to micro-organisms Micro-organisms are «opsonised» (from the Greek word meaning 'to prepare for the table'), or rendered more amenable to phagocytosis. Opsonins are freely circulating serum molecules, which are produced to attach to the surface of microbes, so rendering them more attractive to phagocytes. The process of coating a particle with opsonins is called opsonization. Examples of opsonins include:

- IgG antibody - part of the immune response,
- C3b molecule of the complement system.

Each has receptors for both foreign particle and host phagocyte. In the immune individual, the binding of immunoglobulins to microorganisms by their Fab components leaves the Fc component exposed. Neutrophils have surface receptors for

the Fc fragment of immunoglobulins, and consequently bind to the microorganisms prior to ingestion.

Bacterial lipopolysaccharides (LPS) activate complement components via the alternative pathway, generating component C3b that has opsonizing properties. In addition, if antibody binds to bacterial antigens, this can activate complement via the classical pathway; also generating C3b. Opsonisation can itself stimulate the local activation of complement, further enhancing the local production of C3b opsonin and phagocytosis. Microorganisms have produced a myriad of ways of preventing opsonization, for example, Staphylococcal alpha toxin, an exotoxin, binds to the Fc region of antibody, so preventing binding of phagocyte with the opsonin.

Intracellular killing of microorganisms. Neutrophils are highly specialized cells, containing noxious microbial agents. The microbicidal mechanisms may be classified as:

- oxygen-dependent (oxidative),
- oxygen-independent (nonoxidative).

Oxygen-dependent mechanisms. Prior to adherence to the target microorganism, the phagocyte begins to consume oxygen. This is called the «respiratory burst» or «metabolic burst», and in the neutrophil, very little of this consumed oxygen is used for energy needs. Instead, oxygen is consumed to form reduced oxygen metabolites which will be used to kill the target microbe. The oxidative antibacterial effects are mediated by two main biochemical entities:

- NADPH oxidase system, which spans the cytoplasm and plasma membrane,
- myeloperoxidase (MPO), which localizes into the azurophil granules.

The NADPH oxidase system initiates oxygen reduction. The phagocyte is capable of adding one or two electrons (the electrons originally came from NADPH) to dioxygen (O2) to form superoxide anion (•O2-), hydrogen peroxide (H2O2) and hydroxyl radical (•OH). Most •O2- spontaneously or catalytically (catalysis using an enzyme "superoxide dismutase") dismutates to H2O2 and dioxygen (O2).

The neutrophils produce hydrogen peroxide which reacts with myeloperoxidase in the cytoplasmic granules in the presence of halide, such as Cl, to produce a potent microbial agent. MPO usually oxidizes chloride because of the concentration of chloride in the host tissue fluids. Bactericidal activity of MPO is based upon the formation of hypochlorous acid, which oxidize or chlorinate a variety of structures, especially amines and sufhydryls found in proteins. The end result is the destruction of important bacterial proteins. The bactericidal activity of MPO is dependent upon two functions. First, the phagocyte must be able to form sufficient substrate H2O2 via the respiratory burst pathway. Thus, the phagocyte must be in the presence of dissolved dioxygen. Second, the phagocyte must be able to secrete the MPO into the vicinity of the microorganism, therefore, the phagocyte must be capable of phagosome-lysosome fusion.

Oxygen-independent mechanisms. There are many components within the phagocyte granules (lysosomes) which exert antimicrobial effects. Azurophil granules contain myeloperoxidase; defensins; members of the neutral serine protease family (including elastase, proteinase 3, azurocidin, and cathepsin G); lysozyme (muramidase); bactericidal/permeability increasing protein. Specific granules contain lactoferrin, which chelates iron required for bacterial growth; lysozyme... Azurophil granules are primarily designed for killing microorganisms, specific granule components may kill some organisms as well as solubilize collagen.

Lysis and digestion of bacteria proceeds most rapidly intraphagolysosomally. The neutrophil is not as good at digestion of foreign particles as macrophage, thus, when neutrophil egress from a local site is obstructed, macrophage are always recruited to eliminate neutrophil and bacterial debris. Usually, the neutrophil is eliminated in the form of pus, thereby minimizing the amount of macrophage activity required.

Release of lysosomal products from the cell damages local tissues by proteolysis by enzymes such as elastase and collagenase, activates coagulation factor XII, and attracts other leukocytes into the area. Some of the compounds released increase vascular permeability.

EXUDATES

1) Serous exudate is a form of exudate where the predominant feature is the production of a serum-like fluid. The fluid may derive from increased capillary permeability, as may occur in the early stages of acute inflammation, or it may result from active secretion by cells lining body cavities. Notably, this type of exudate is characterized with the minimal increase of vascular permeability when compared to other types of the exudates. Moreover, there is very low numbers of cells in these exudates. Therefore, serous exudates resemble transudates.

2) A purulent exudate is an inflammatory fluid with a high concentration of leukocytes, predominantly neutrophils, dead cell matter and inflammatory molecules e.g. cytokines, enzymes. The degree of enzyme proteolysis determines the thickness of the fluid. Clinically, pus is usually confined to the area of inflammation as demarcated by a pustule or abscess enclosed within the fibrous pyogenic membrane. It can assume a yellow, green or white colour. The last is associated with Staphylococcal sp. infection.

A pustule is a pus filled lesion less than 1cm in size, the synonym is «abscess». An abscess is a pus-filled lesion, arbitrarily defined as being less than 1 centimeter in diameter, enclosed within a pyogenic membrane. Abscesses are most frequently caused by staphylococcal infection within the skin. However, abscesses may occur anywhere in the body and the infecting organism is largely determined by the site. Abscesses may be acute or chronic. The latter are typified by the cold abscess of tuberculous infection.

3) Hemorrhagic exudate is an inflammatory fluid with a high concentration of erythrocytes. This type of exudates is due to the significant increase of vascular permeability (Smallpox, Anthrax, Arthus phenomenon).

4) Fibrinous exudate is a form of inflammatory exudate, which is characterized by fibrin deposition. It may be acute, but more often it is a chronic response. It results from the exudation of a high concentration of the plasma protein fraction. There is activation of the coagulation cascade and deposition of fibrin locally. Ultimately, organisation of the fibrin can occur with rigid fibrous tissue being laid down. Body cavities and potential spaces are sites where fibrinous inflammation is more common e.g. the pericardial cavity, potentially resulting in restrictive fibrosis, pleural space, peritoneal cavity, potentially resulting in adhesions.

OUTCOMES OF THE ACUTE INFLAMMATION
Lesson 2

The outcomes of acute inflammation depend upon the type of tissues involved and the amount of tissue destruction, which depend in turn upon the nature of the injurious agent. The possible outcomes of acute inflammation are:

1) resolution
2) regeneration
3) organization also named as a tissue repair
4) supporation
5) chronic inflammation

1) Resolution. Dead cellular material and debris are removed by phagocytosis (mainly by macrophages) and the tissue is left with its original architecture intact.

2) Regeneration. Lost tissue is replaced by proliferation of cells of the same type, which reconstruct the normal architecture.

3) Repair. Lost tissue is replaced by a fibrous scar, which is produced from granulation tissue.

All of these processes may occur in the same tissue, and begin as soon as there is significant tissue damage; healing reactions do not wait for inflammation or other damaging mechanisms to subside, but take place at the same time. The outcome in any particular situation depends on which of the 3 processes predominates (resolution, regeneration and repair), and this in turn depends on a number of factors.

4) Supporation. The formation of pus, a mixture of living, dying and dead neutrophils and bacteria, cellular debris and sometimes globules of lipids.

5) Chronic inflammation. If the agent causing acute inflammation is not removed, the acute inflammation may progress to the chronic stage.

1) Resolution of Acute Inflammation The term resolution means the complete restoration of the tissues to normal after an episode of acute inflammation. The conditions, which favor resolution, are:

- minimal cell death and tissue damage (little tissue destruction),
- rapid destruction of the causal agent (e.g. either naturally - phagocytosis of bacteria or with therapeutic help- antimicrobal agents),

- rapid removal of fluid and debris by good local vascular drainage,
- occurrence in an organ or tissue which has regenerative capacity (e.g. the liver) rather than in one which cannot regenerate (e.g. the central nervous system).

A good example of an acute inflammatory condition, which usually resolves completely is acute lobar pneumonia. In the earlier stages of lobar pneumonia the alveolar spaces fill with exudates, containing fibrin, bacteria and neutrophil polymorphs (pus) but the alveolar walls remain intact. If the infecting organism is successfully destroyed at this stage (either naturally or with therapeutic help) then the purulent material may be completely scavenged from the air spaces by macrophages, leaving the original lung structure intact.

Therefore, the sequence of events leading to resolution is usually:

- phagocytosis of bacteria (e.g. pneumococci) by neutrophils and intracellular killing,
- fibrinolysis,
- phagocytosis of debris, especially by macrophages, and carriage through lymphatics to the hilar Iymph nodes,
- disappearance of vascular dilatation,
- following this, the parenchyma would appear histologically normal.

2) Regeneration of Acute Inflammation The capacity of various cells to regenerate is a constitutive-dependent feature.

Cell type: Cells are usually classified into three groups depending on their capacity for regeneration. Labile cells are those, which normally have a high rate of loss and replacement (e.g. squamous and glandular epithelia, haemopoeitic cells in bone marrow) and therefore have a high capacity for regeneration. Stable cells do not normally proliferate to a significant extent but can be stimulated to do so after damage. Examples include renal tubular cells, hepatocytes, osteoblasts, endothelial cells, fibroblasts. Permanent cells are unable to divide after initial development and therefore cannot regenerate when some are lost. The best example here is neurons.

Tissue architecture: Simple structures are easier to reconstruct following damage than complex ones. For example a flat surface such as epidermis regenerates very successfully, but dermal sweat glands do not. An imperfect attempt at regeneration of tissue architecture may have important clinical consequences: for example, in some chronic inflammatory liver diseases regenerative proliferation of hepatocytes is very vigorous, but damage to the connective tissue framework of the liver tissue means that the regenerated tissue has an abnormal nodular architecture - cirrhosis. The abnormal architecture leads to haemodynamic abnormalities in the hepatic portal venous system - portal hypertension.

Amount of tissue loss: The idea of regeneration implies that there are cells left to regenerate. For example, if there is loss of a large area of epidermis then its central regions will heal by scar formation rather than regeneration, since the rate of migration of new epidermal cells from the edges of the wound is limited and scarring will proceed before they are able to cover the damaged area.

3) Organization of tissues is their replacement by granulation tissue. The term granulation tissue is derived from the appearance of small, red, granular foci which bleed easily, commonly seen within freshly healing tissue. These can be readily demonstrated in the base of skin wounds when the overlying scab is picked off. The granulations consist of clusters of fragile newly-formed capillary blood vessels which proliferate and grow into damaged tissue along with fibroblasts at an early stage of the repair process.

The circumstances favoring this outcome of the acute inflammation are when:

- Iarge amounts of fibrin are formed, which cannot be removed completely by fibrinolytic enzymes from the plasma or from neutrophil leukocytes,
- substantial volumes of tissue become necrotic or if the dead tissue (e.g. fibrous tissue) is not easily digested,
- exudate and debris cannot be removed or discharged.

During organization, new capillaries grow into the inert material (inflammatory exudate), macrophages migrate into the zone and fibroblasts proliferate, resulting in fibrosis. A good example of this is seen in the pleural space following acute lobar pneumonia. Resolution usually occurs in the lung parenchyma, but very extensive fibrinous exudate fills the pleural cavity. The fibrin is not easily removed and consequently capillaries grow into the fibrin, accompanied by macrophages and fibroblasts (the exudate becomes 'organized'). Eventually, fibrous adhesion occurs between the parietal and visceral pleura.

The steps in the organization are as follows:

- Phagocytosis of necrotic debris and other foreign material by macrophages.
- Proliferation of blood vessel endothelial cells and fibroblasts at the edges of the damaged area.
- Endothelial cells grow into the damaged area, initially as solid buds from these adjacent blood vessels, the solid buds then canalise to form an abundant network of delicate, thin-walled capillaries.
- Fibroblasts migrate into the damaged area along with the capillaries to form a loose connective tissue framework, this delicate fibrovascular tissue is granulation tissue.
- The new capillary vessels anastomose to establish a blood circulation in the healing area and differentiate towards arterial and venous types as necessary, fibroblasts produce collagen, giving the healing tissue mechanical strength.
- Eventually a mature scar consisting almost entirely of dense collagen is produced.

It is a general rule that the volume of scar tissue produced is always less than the bulk of the tissue it is replacing. This can have important clinical consequences where such scar contraction distorts the tissue enough to interfere with function. For example,

scarring of tubular structures such as the intestines can produce stenosis of the lumen and obstruction; scarring of the skin around a joint can produce contractures and immobility. The above stages of scar formation do not have to occur in strict sequence and different parts of a developing scar are in general at different stages at any given moment.

Wound healing

The time course of healing by repair and the amount of scar tissue formed depend on factors such as the extent of tissue damage, presence of persisting infection, inflammation, etc. The relatively simple, rapid process of healing in a clean skin wound which has been closed promptly and where tissue damage is minimal (e.g. a surgical incision) is termed healing by primary intention. In this situation the epidermis regenerates across the gap quickly and successfully, the volume of tissue into which granulation tissue has to grow is small, and the amount of fibrous scar produced is minimal.

Healing of an open wound where there is significant tissue loss, or where there is ongoing tissue damage from infection is termed healing by secondary intention. In this situation the amount of granulation tissue formed may be substantial, scar contraction much greater, and re-epithelialisation less complete.

Factors influencing healing. The rate of healing and the success of formation of scar tissue can be limited by many adverse factors. Some of the local and systemic factors, which are of importance clinically.

Local:

- Persisting infection, foreign material or other stimulus to inflammation.
- Inadequate blood supply.
- Excessive movement.
- Irradiation.
- Locally applied drugs, e.g. corticosteroids.

Systemic:

- Age: the healing process becomes slower and less effective with age.
- Nutritional deficiencies, e.g. vitamin C, zinc, protein.
- Metabolic diseases, e.g. renal failure, diabetes mellitus.
- Catabolic state associated with malignancies.
- Systemic drugs, e.g. corticosteroids.

4) Suppuration is the formation of pus. The causative stimulus must be fairly persistent and is virtually always an infective agent, usually pyogenic bacteria (e.g. Staphylococcus aureus, Streptococcus pyogenes, Neisseria species or coliform organisms), sometimes viruses, chemicals. Once pus begins to accumulate in a tissue, it becomes surrounded by a 'pyogenic membrane' consisting of sprouting capillaries,

neutrophils and occasional fibroblasts. Such a collection of pus is called an abscess, and bacteria within the abscess cavity are relatively inaccessible to antibodies and to antibiotic drugs (thus, for example, acute osteomyelitis, an abscess in the bone marrow cavity, is notoriously difficult to treat).

Abscess pathogenesis. An abscess starts around a bacterial focus. The origin of the infective agent is extremely varied e.g. puncture wounds of the skin, haematogenous spread from a primary infection, perforation of the gastrointestinal tract.

- The inflammatory response is initiated, neutrophils are attracted to the area by chemotactic products.
- Neutrophils are overwhelmed by infection, die, and release proteolytic enzyme products.
- Platelets are activated and release growth factors, these in turn stimulate fibroblasts and blood vessels.
- Fibrin and collagen are laid down at the periphery and angiogensis occurs; this vascular fibrous layer, rich in cells like polymorphs and monocytes, is termed the pyogenic membrane. The pyogenic membrane physically inhibits bacterial advancement but also hinders the penetration of antibiotics.
- Within the abscess cavity, proteolytic destruction of cells and matrix structures leads to the production of more chemotactic factors. Consequently, more neutrophils and macrophages ingress into the centre of the abscess where liquefactive necrosis is occurring.
- Once the bacteria have reached a maximal concentration within the pus - dependent on the individual bacterium - they no longer proliferate. This makes them even more insensitive to antibiotics.
- If the abscess does not drain by surgical intervention or tracking along tissue planes, macrophages begin to replace neutrophils as the dominant population of cells within the pyogenic membrane.
- The membrane eventually becomes less vascular and contracts to leave a scarred fibrous sheet that may become calcified. The contents are resorbed by liquefaction and reabsorption of water.

An increasingly large abscess can press on external blood vessels causing thrombosis and necrosis in its path. This process accounts for its ability to track through tissue.

An abscess usually 'points', then bursts; the abscess cavity collapses and is obliterated by organisation and fibrosis, leaving a small scar. Sometimes, surgical incision and drainage is necessary to eliminate the abscess. If an abscess forms inside a hollow viscus (e.g. the gall bladder) the mucosal layers of the outflow tract of the viscus may become fused together by fibrin, resulting in an empyema.

Such deep-seated abscesses sometimes discharge their pus along a sinus tract (an abnormal connection, lined by granulation tissue, between the abscess and the skin or a mucosal surface). If this results in an abnormal passage connecting two mucosal

surfaces or one mucosal surface to the skin surface, it is referred to as a fistula. Sinuses occur particularly when foreign body materials are present, which are indigestible by macrophages and which favor continuing suppuration. The only treatment for this type of condition is surgical elimination of the foreign body material.

5) Progression to Chronic Inflammation.

Secondarily chronic. If the agent causing acute inflammation is not removed, the acute inflammation may progress to the chronic stage. Deficient activity of inflammatory cells and mediators may be caused by chronic stress, poor lifestyle habits and environmental pollutants, preexisting infection, medications. In addition to organization of the tissue just described, the character of the cellular elements changes, with lymphocytes, plasma cells and macrophages replacing the neutrophil polymorphs.

Primarily chronic. Often chronic inflammation occurs as a primary event, they're being no preceding period of acute inflammation. Researchers now recognize another kind of inflammation: silent inflammation, or SI.

The chronic and continuous low-level demand that silent inflammation places on the body's defense systems results in an immune-system breakdown. The body tissues themselves may lose their ability to recognize cells that are «self» from «nonselft», and the body may mistakenly identify its own cells as foreign invaders. This internal programming error then continues to trigger and retrigger immune responses, setting the stage for autoimmune diseases, such as lupus, multiple sclerosis, rheumatoid arthritis and scleroderma. The result is that this process may be happening year after year without our even being aware of it.

The factors initially inducing the chronic inflammation are:

a) Intracellular pathogens, e.g. Mycobacteria, Brucellae, viruses (chronic viral hepatitis), etc.

b) Large extracellular pathogens like many fungi (Histoplasmosis, Blastomyces, Cryptococcosis, Coccidioides), parasites.

c) Prolonged exposure to toxins or mechanical irritation such as silica, asbestos etc.

d) Autoantigens inducing autoimmune diseases such as rheumatoid arthritis, lupus, multiple sclerosis etc

3 main components of chronic inflammation are:

- Mononuclear cell (macrophages, lymphocytes) infiltration.
- Tissue destruction caused by reactive oxygen species, proteases, tissue plasminogen activator (tPA).
- Repair by fibrosis caused by cytokines like PDGF, FGF, TGFβ, VEGF, etc.

The conditions, which favor chronicity, are:

- Macrophages live far longer than neutrophils, which last for a few days at most. Because they are long-lived, indigestible material may remain inside macrophages in vesicles for long periods.
- Macrophages are important secretory cells releasing damaging substances – oxygen metabolites, proteases; and inflammatory mediators – PG, LT, growth factors, proinflammatory cytokines IL-1, IL-6 and TNFα.
- Macrophages are avid phagocytes, and, even if they can't digest all the material phagocytized, they will continue to engulf more.
- In acute inflammation, removal of the stimulus halts the recruitment of monocytes into the inflamed tissue, and existing macrophages exit the tissue via lymphatics. In chronically inflamed tissue the stimulus is persistent, and therefore recruitment of monocytes is maintained, existing macrophages are tethered in place, and proliferation of lymphocytes is stimulated.
- Accumulation of macrophages and lymphocytes in areas of chronic inflammation occurs in three ways: continued recruitment from the circulation, local proliferation, prolonged survival and immobilization in the inflamed area.

Thus, chronic inflammation is a pathological condition characterized by concurrent active inflammation, tissue destruction, and attempts at repair. Chronic inflammation is not characterized by the classic signs of acute inflammation, mainly due to exudation. Instead, chronically inflamed tissue is characterized by the infiltration of mononuclear immune cells (monocytes, macrophages, lymphocytes, plasma cells, fibroblasts), tissue destruction, and attempts at healing, which include angiogenesis and fibrosis.

Histological appearances in chronic inflammation. The microscopic appearances of chronic inflammation vary considerably according to the site involved and the causative stimulus. However, the general features are:

- A mixed inflammatory cell infiltrate containing macrophages, lymphocytes, plasma cells, fibroblasts with neutrophil and eosinophil polymorphs as possible minor components.
- Macrophages are predominant cells in nonimmune-initiated cases; lymphocytes are predominant cells in the immune-initiated cases.
- CD4 T helper lymphocyes are crucial in recruiting monocytes which turn into macrophages. Lymphoid cells can proliferate at the site of inflammation as well as in local lymph nodes; in severe cases this can give rise to lymphoid follicles with germinal centers in the inflammatory lesion.
- Tissue destruction (necrosis) caused both by the causative agent and by the inflammatory process itself.
- Attempts at reconstructing the damaged tissue occur simultaneously with the inflammatory process. These can be considered under the general title of healing

and repair. The attempts at reconstruction may have different outcomes. If there is little tissue destruction then some organs may be able to regenerate their original structure, or mild inflammation may terminate by resolution without causing any structural damage. Commonly, however, the original structure cannot be re-created and the damaged area undergoes fibroblasts activation, collagen production, ending up with a dense fibrous scar.

Granulomatous inflammation (Table 1) is a histologically distinctive form of chronic inflammation that occurs in particular circumstances in response to certain organisms or foreign material. This term (granuloma, granulomatous inflammation) is not to be confused with granulation tissue.

Table 1. Examples of granulomatous inflammation

Specific Infections:	Mycobacteria (tuberculosis, leprosy, others), syphilis, brucellosis, fungi, parasites (e.g. Schistosoma).
Foreign bodies:	Endogenous: e.g. keratin, necrotic boneor adipose tissue, uric acid crystals (gout). Exogenous: e.g. wood, grit, silica or asbestos dust, talc, suture material, silicone, prostheses.
Specific chemicals:	Beryllium.
Drugs:	Hepatic granuloma due to allopurinal, phenylbutazone, sulphonamides.
Unknown:	Sarcoidosis, Crohn's disease.

Structure of a granuloma. Granulomas are aggregates of particular types of chronic inflammatory cells, which form nodules in the millimeter size range. Granulomas may be confluent, forming larger areas.

The essential components of a granuloma are collections of modified macrophages, termed epithelioid cells, usually with a surrounding zone of lymphocytes. Epithelioid cells are so named by tradition because of their histological resemblance to epithelial cells, but are not in fact epithelial; they are derived from blood monocytes, like all macrophages. Epithelioid cells are less phagocytic than other macrophages and appear to be modified for secretory functions. The full extent of their functions is still unclear.

Macrophages in granulomas are commonly further modified to form multinucleate giant cells. These arise by fusion of epithelioid macrophages without nuclear or cellular division forming huge single cells, which may contain dozens of nuclei. In some circumstances the nuclei are arranged round the periphery of the cell, termed a Langhans-type giant cell (characteristically seen in tuberculosis); in other circumstances the nuclei are randomly scattered throughout the cytoplasm - for

example in the foreign body type of giant cell which is formed in response to the presence of other indigestible foreign material in the tissue.

Areas of granulomatous inflammation commonly undergo necrosis. The prototype example here is caseous necrosis in tuberculosis. Formation of granulomatous inflammation seems to require the presence of:

- indigestible foreign material (derived from bacteria or other sources)
- a cell-mediated immune reaction against the injurious agent (type IV hypersensitivity reaction).

The finding of granulomatous inflammation in a biopsy specimen can be very useful in limiting the number of possible causes of the inflammatory process.

FEVER

Fever is an elevation of body temperature above the normal circadian variation. The normal body temperature varies with the day. If it is >37.2^0 C (98.9^0 F) at 6 AM and >37.7^0 C (99.9^0 F) at 4 PM, it is considered as fever (Table 2).

Table 2. Body temperature: The normal and the abnormal

Temperature	^{0} Centigrade	^{0} Faranheat
Normal	36.6 - 37.2^0 C	98 - 99^0 F
Pyrexia	>37.2^0 C	>99^0 F
Hyperpyrexia	>41.6^0 C	>107^0 F
Subnormal	<36.6^0 C	<98^0 F
Hypothermia	<35^0 C	<95^0 F

The thermoregulatory center is located in the hypothalamus, it involves a conglomeration of nuclei in the diencephalon of the brain. Its arrangement and function are very similar to that of the thermostatically controlled household furnace.

In the hypothalamus a pre-determined ideal core body temperature for homeostasis is sent to a comparing device along with the actual core body temperature that is picked up by a temperature sensor. If there is a significant discrepancy, an error signal of too hot or too cold is relayed to an antirise center or antidrop center respectively (Fig. 8).

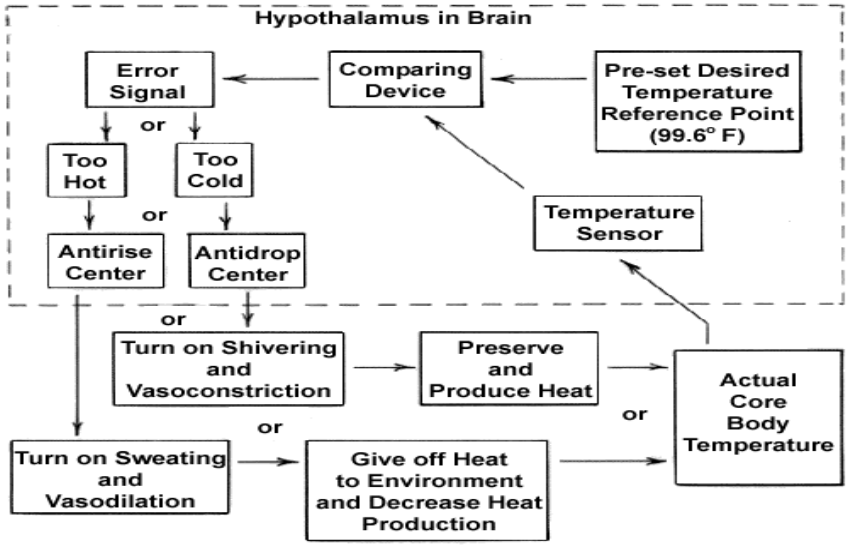


Figure 8. Thermoregulation in the hypothalamus

If the antirise center is stimulated, it kicks on the sweating and vasodilation mechanisms, which release heat to the environment and decrease heat production in the body.

If the anti-drop center is stimulated, it kicks on the shivering and vasoconstriction mechanisms which preserve heat and produce heat in the body. At a central thermointegrative level, fever is a consequence of elevation of the homeostatic temperature set point within the hypothalamus. The posterior hypothalamus discriminates a difference between the set point and core body temperature. It then activates the normal physiological mechanisms to elevate temperature via the anterior hypothalamus, so reducing the difference.

Etiology

Substances that cause fever are called pyrogens.

- Exogenous pyrogens are mostly derived from microbes, microbial products or toxins, such as gram-negative endotoxin (bacterial lipopolysaccharides - LPS) or the toxin from Staphyllococcus aureus.
- Endogenous pyrogens are cytokines (polypeptides) produced in particular by monocyte macrophages but also by skin, endothelial, epithelial and glial cells. The cytokines known to cause the elevation of body temperature are: TNF-α, IL-1, IL-6, interferon α, interferon β. They are termed «endogenous» pyrogens because they derive form endogenous sources rather than form bacterial components.

Molecular mechanisms

The molecular stimulus for resetting of the hypothalamic set point for temperature has been partly elucidated for infective and inflammatory states. Bacterial products, particularly lipopolysaccharides (LPS), upon interaction with cells of the mononuclear phagocytic system, cause release of an endogenous pyrogen molecule from the phagocyte, interleukin-1, 6. Alternatively, inflammatory cells may release interleukin-1 and 6 and TNF-α in response to generalized inflammation.

These cytokines pass haematogenously to the preoptic area of the hypothalamus. At this site, there is thought to be an intermediate molecule, which is stimulated by the cytokine to cause pyrexia. This was believed to be a prostaglandins PGE1, PGE2 (due to the action of aspirin in reducing fever; evidence is equivocal). Endogenous pyrogens cause the hypothalamus to reset the body's thermostat and bring into play mechanisms that raise and maintain a higher than normal temperature. Shivering and rigors are part of this mechanism.

Shivering, rigors, chills. Pyrogens cause the hypothalamus to raise the body's temperature by creating additional heat and reducing heat loss. Shivering (chill) is triggered to generate heat from muscle activity. Chills are caused by rapid muscle contraction and relaxation, and are the body's way of generating heat. A rigor is an episode of shaking or exaggerated shivering which can occur with a high fever.

Peripheral vasoconstriction reduces heat loss by shunting blood internally, hence making the patient look pale. These mechanisms continue until the blood reaching the hypothalamus reaches the new setting. The hypothalamus maintains the raised temperature at the new setting until a lower level is set when sweating, vasodilation and the evoparation of insensible fluid loss achieve heat loss.

Sweating. is a very non-specific symptom, and may be physiological or pathological. Sweat is formed by active secretion deep within the dermis. Its composition is initially near to that of plasma but with minimal protein. During passage along the sweat duct, reabsorption of sodium and chloride ions occurs until their concentrations are less than half of their plasma values e.g. 60 mmmol and 50 mmol respectively. Conversely, potassium is secreted to give a value of 7-8 mmol in sweat.

The control mechanism for sweat secretion is both neural – cholinergic fibres of the thoracolumbar sympathetic nervous system descending from the anterior hypothalamus and hormonal – adrenaline. The observation that local and systemic administration of atropine (a muscarinic-receptor antagonist) greatly attenuates or abolishes sweating during a thermal challenge or during exogenous administration of acetylcholine or its analogs strongly suggests that thermoregulatory sweating primarily occurs through stimulation of muscarinic receptors.

When the rate of sweat production is elevated, as occurs during exercise or heat stress, ion reabsorption mechanisms can be overwhelmed due to the large quantity of sweat secreted into the duct, resulting in higher ion losses. Thus the sodium content in sweat on the skin’s surface is greatly influenced by sweat rate. If the rate of production is high, excessive amounts of solute may be lost and so aldosterone stimulation is

triggered. As well as its renal reabsorption role, it also enhances sodium and chloride reabsorption along the duct of the sweat gland.

Thermoregulatory sweating Sweating, along with vasodilatation, is one of the body's means of reducing temperature. The signal to increase the rate of sweating arises from the hypothalamic sympathetic outflow to exocrine sweat glands. Heat is lost from the surface of the skin as the energy used to evaporate sweat. The evaporation of one gram of water uses 2.4kJ of heat. Typically, every hour there is a basal, insensible evaporative loss of water of about 20-30 grams from both skin and lungs. The rate of sweating is dependent upon core as well as surface body temperature. It occurs at a higher core temperature if the surface temperature is lowered, and vice versa. The capacity of sweating to reduce temperature is dependent upon external humidity. Hence, in high humidity environments e.g. rain forest, a lower temperature is tolerated than low humidity settings e.g. desert. Hyperhidrosis is the excessive production of sweat.

Fever response. Fever is a rise in body temperature above the normal daily variation, greater than 37.8'C orally or 38.2'C rectally. There is a diurnal variation, lowest in the early morning and highest in the late afternoon and evening ('teatime to bedtime'). It may be caused by infection, inflammation, neoplasm or an immunologically mediated disorder.

The pattern of fever may be:

a) intermittent (or named as undulant fever) (swings between raised and normal; disorder of values between 1- 3 C; temperature returns to normal or may be even below normal in the morning),

b) remittent (swings between morning and evening; disorder of values is between 1,5 and 2 C ; the temperature does not return to normal,

c) exhausting (or hectic) (swings between morning and evening, may not return to normal (as remittent), but (in contrast to remittent) disorder of values is much higher (between 3 and 5 C),

d) relapsing (or recurrent)(after the fever-free period the temperature raise again).

The patterns of fever may have the diagnostic value.

1) Intermittent fever is observed in malaria. P. malariae causes fever every 4th day, ie 1,4,7. Relapse occurs because the parasite which lays dormant in either the liver (the exoerythrocytic schizogony) or in the blood (the erythrocytic schizogony). After the propagation of the P. malariae inside erythrocytes they become released from erythrocytes, which in turn leads to erythrocyte lysis (hemolysis). The fever reaction correlates with the massive release of P. malariae from erythrocytes and reflect the reaction of human body to massive foreign protein invasion.

2) Hectic fever is observed in sepsis syndrome (also named as septicaemia), which is known to be associated with the presence and persistence of pathogenic organisms or their toxins in the blood. Since in septicaemia the bacteria growth is excessive (in contrast to bacteraemia – no bacteria growth), the fever occurs as a

sequence of the significant increase of the bacteria products containing exogenous pyrogens after the bacteria become destroyed .

3) Remittent fever is usually observed in tuberculosis.

Fever effects on the whole body.

Beneficial effects:

- increased metabolism,
- increased blood supply to organs (required for complete regeneration),
- decrease of bacteria growth (bacteriostatic effect), killing the microorganisms (bacteriolytic effect),
- increased antibody, INF-γ synthesis (antibacterial, antiviral resistance),
- enhanced neutrophil production and T-lymphocyte proliferation,
- aids in the body's acute-phase reaction,
- decrease of Fe level in serum (required for bacteria growth).

Harmful effects:

- risk of febrile convulsions in children,
- in adults with cardiac and pulmonary failure can trigger the possibility of collapse development during critical decrease of fever,
- increased insensible water losses,
- acid-base disorders: metabolic acidosis in mild cases, respiratory alkalosis in severe cases.

Pyrotherapy: Pyrotherapy represent the therapy of diseases by elevating of body temperature or sustaining an elevated body temperature. It is useful in as a component of treatment of: 1) chronic diseases (tuberculosis, syphilis etc.); 2) diseases with inadequate blood supply, which induce the function failure (e.g. renal disorders), 3) cancer chemotherapy.

Antipyretics: Prostaglandin E2 plays a critical role in producing fever. Drugs that inhibit brain cyclo-oxygenase are effective in reducing fever, most often used being paracetamol, aspirin and other NSAID's. Experimental evidence suggests that host defense mechanisms are enhanced by a raised temperature. Antipyretics should perhaps not be used routinely but clearly have a use for children at risk of febrile convulsions, for adults with cardiac and pulmonary failure (to reduce excessive O2 demand), and where fever causes delirium.

ALLERGY

The term “hypersensitivity reaction” underlines the harmful immune response that produces the tissue injury. These are an excessive, undesirable (damaging, and

sometimes fatal) reactions are produced by the immune system. Hypersensitivity reactions require a pre-sensitized (immune) state of the host. Frequently, a particular clinical condition (disease) may involve more than one type of reactions.

The original classification of the hypersensitivity reactions was initially defined by Gell and Coombs. They classified hypersensitivity reactions as four different types.

- Type I – IgE mediated; immediate hypersensitivity reaction
- Type II – cytotoxic hypersensitivity reaction
- Type III – immune complex mediated reactions
- Type IV – delayed-type hypersensitivity reactions (in this form of hypersensitivity the immune response is mediated by the action of the lymphocytes rather than by antibody).

Type I hypersensitivity reactions. Mechanisms and etiology.

It is currently accepted that type I hypersensitivity reactions are caused by the interaction of mast cell-bound IgE with allergen molecules. The resulting mast cell degranulation releases inflammatory mediators, including histamine and arachidonic acid metabolites.

Evidence supporting an important role of IgE in type I hypersensitivity reactions is also based on the passive cutaneous anaphylaxis model, where IgE-containing serum may transfer allergen-specific hypersensitivity to the skin. However, in was also recently shown that genetically engineered mice, deficient of IgE, are also susceptible to anaphylaxis. New models of passive cutaneous anaphylaxis propose an alternative IgG_4-mediated pathway for type I hypersensitivity reactions.

Type I hypersensitivity reactions (IgE-mediated) are a rapid allergic reaction to specific antigens found in, e.g. house dust, pollen and animal danders. The symptoms and signs of the reaction are apparent within 30 min of the exposure. Atopic individuals are susceptible to type I hypersensitivity reactions.

There are three steps of the IgE-mediated allergic response:

Step 1 (sensitization): Initial exposure to allergen leads to production of allergen-specific IgE. Features of allergens, favoring the IgE production are low dose and molecular weight and transmucosal delivery. There are following events in the step 1 of the IgE-mediated response. Antigen-presenting cells (APCs) (e.g. dendritic cells and macrophages) recognize and process the antigen (Ag) antigenic peptides. APCs present a peptide portion in the context of MHC class II molecules to T lymphocytes, which in turn stimulate the secretion of cytokines from T-lymphocytes. Direct interaction of peptide-presenting T-lymphocyte and B-lymphocyte stimulates antigen-specific IgE production by the B-lymphocyte. Secreted IgE is then bound by high-affinity receptor type I (FcεRI) on mast cells and/or basophils and low-affinity receptor type II (FcεRII) on eosinophils, platelets, lymphocytes.

Step 2 (early phase reaction). Occurs within the minutes of subsequent repeated exposure to the allergen with the following formation of the immune complexes (IgE-antigen) on the surface of the mast cells and other cell types (expressing the FcεRI and

FcεRII). The formation of immune complex on the surface leads to the cell activation with the following mediators release. Therefore, there are following events in step 2 of the IgE-mediated response. Upon re-exposure to allergen, mast cells and basophils degranulate, releasing pre-formed mediators such as histamine, tryptase and heparine, and newly synthesized mediators such as leukotrienes (LTs) and prostaglandins (PGs), as well. Histamine is the most thoroughly studied mediator of the early allergic response, producing:

- smooth muscle constriction,
- mucus secretion,
- increased vascular permeability,
- nerve stimulation.

Step 3. (late phase reaction). Occurs within hours to days of the repeated allergen exposure and reflects the influx of inflammatory cells (eosinophils, neutrophils and basophils). There are following events in the step 3 of the IgE-mediated response. Allergen stimulates the cells (e.g. mast cells, T-cells) to produce the newly-formed inflammatory mediators (e.g. luekotrienes, prostaglandines, cytokines). The newly-formed mediators act at the post-capillary endothelial cells, promoting:

- outflow of plasma leading to the localized edema,
- adhesion of the circulating leukocytes,
- tissue infiltration by eosinophils, neutrophils and basophils.

Over the course of several hours, the infiltrating inflammatory cells become activated and release mediators stimulating and enhancing further inflammatory reactions: Eosinophils produce mediators (LTs, major basic protein (MBP), eosinophil cationic protein (ECP) that promote the tissue damage associated with chronic allergic reactions. Th2-lymphocytes release cytokines that promote: 1) IgE production, 2) eosinophil chemoattraction; 3) increased numbers of mucosal mast cells

Type I reaction may involve skin (urticaria and eczema), eyes (conjunctivitis), nasopharynx (rhinorrhea, rhinitis), bronchopulmonary tissues (asthma) and gastrointestinal tract (gastroenteritis), or the whole body (anaphylactic shock)

Type II hypersensitivity

Type II hypersensitivity (cytotoxic hypersensitivity) reaction is characterized by antibodies directed toward antigens that are present on cell surfaces outside the cells. The antigens can either be from the body itself or from outside the body (for example, bacteria or microorganisms that infect the body), exogenous chemicals – haptens, which can attach to cell membranes can also lead to type II hypersensitivity. In contrast to type I hypersensitivity reactions, type II hypersensitivity reactions are accomplished with activation of complement.

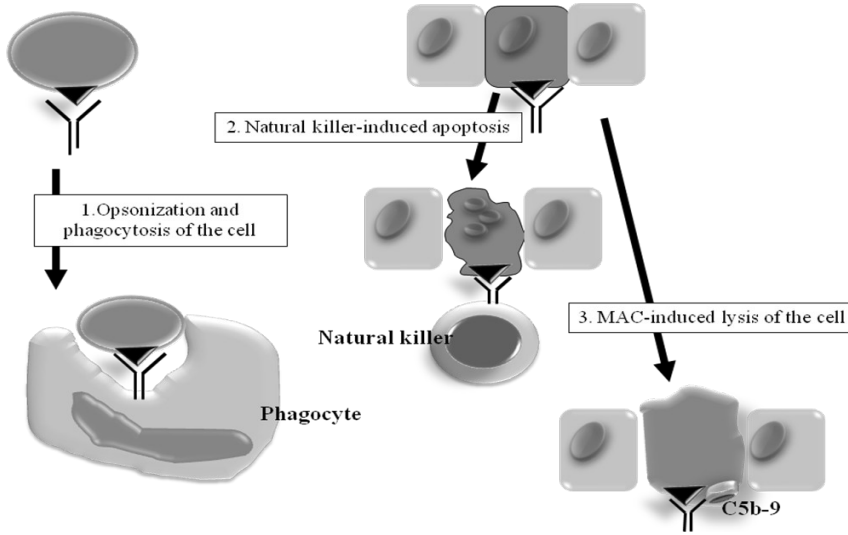


Figure 9. Mechanisms of antibody-dependent cell-mediated cytotoxicity

Basic events in this form of hypersensitivity are:

- the IgG or IgM antibodies are produced in response to challenge with an antigen,
- antibodies bind to the surface of antigen-bearing cellular elements (circulating or fixed),
- IgG and IgM antibodies and antigens form complexes that activate the classical pathway of complement to eliminate cells presenting foreign antigens,
- as antibodies react with a host cell surface, tissues damage result,
- tissue injury involves also natural killer cells and macrophages in antibody-dependent cell-mediated cytotoxicity (ADCC).

Mechanism of ADCC (Fig. 9): Either IgG or IgM is made against self antigens as a result of a failure in immune tolerance, or a foreign antigen resembling some molecule on the surface of host cells enters the body and IgG or IgM made against that antigen then cross reacts with the host cell surface.

The binding of these antibodies to the surface of host cells then leads to:

- Opsonization of the host cells whereby phagocytes stick to host cells by way of IgG, C3b, or C4b and discharge their lysosomes
- Activation of the classical complement pathway causing MAC lysis of the cells
- NK cells attach to the Fc portion of the antibodies, the NK cell then release pore-forming proteins called perforins and proteolytic enzymes called granzymes.

Granzymes pass through the pores and activate the enzymes that lead to apoptosis of the target cell by means of destruction of its structural cytoskeleton proteins and by chromosomal degradation.

Type II reaction may involve AB and Rh blood group reactions; autoimmune diseases such as: rheumatic fever where antibodies result in joint and heart valve damage; idiopathic thrombocytopenic purpura where antibodies result in the destruction of platelets; myasthenia gravis where antibodies bind to the acetylcholine receptors on muscle cells causing faulty enervation of muscles; Goodpasture's syndrome where antibodies lead to destruction of cells in the kidney; multiple sclerosis where antibodies are made against the oligodendroglial cells that make myelin, the protein that forms the myelin sheath that insulates the nerve fiber of neurons in the brain and spinal cord; and some drug reactions.

Antibody-mediated destruction of red blood cells (hemolytic anemia) or platelets (thrombocytopenia) is a side-effect associated with intake of certain drugs such as the antibiotics (penicillin), the anti-cardiac arrythmia drugs (quinidine), antihypertensives (methyldopa). Drug bind to the cell surface and serves as a target for anti-drug IgG antibodies that cause the destruction of the cell. The anti-drug antibodies are made in only a minority of individuals. The cell-bound antibody triggers clearance of the cell from circulation, predominantly by tissue macrophages in the spleen, which bear Fcγ receptors.

The hypersensitivity reaction can take place intravascularly. The physiological example of this type reaction represents adverse reactions to blood transfusions.

ABO incompatibility describes an immune reaction that occurs in the body if two blood samples of different, incompatible ABO types are mixed together. A, B, and O are the three major blood types. The types are defined by molecules on the surface of the blood cells (Table 3). In people of different blood types, these molecules act as antigens, each person has a combination of two of these surface molecules. Type O refers to a lack of any molecule. So, the resulting types are type A (AA or AO molecules), type B (BB or BO molecules), type AB, or type O. People of a given type form antibodies against other types.

Table 3. ABO blood types

Genotype	Red cell Ags	Blood type	Serum Abs (haemolysins)
OO	None	O (I)	AB
AO	A	A (II)	B
AA	A	A (II)	B
BO	B	B (III)	A
BB	B	B (III)	A
AB	AB	AB (IV)	None

When exposed to another type of blood, a breakdown reaction occurs.

- A patient with type A blood will react against type B or type AB blood.
- Similarly, a patient with type B blood will react against type A or type AB blood.
- Patients with type O blood will react against type A, type B, or type AB blood.

Because type O signifies a lack of any surface molecules, type O blood does not cause an immune response. This is why type O blood cells can be given to patients of any blood type, and people with type O blood are called "univeral donors." However, people with type O can only receive type O. Since antibodies are present in the blood plasma, plasma transfusions as well as whole blood transfusions must be matched to avoid causing an immune reaction.

ABO incompatibility problems arise in 2 situations: 1. blood transfusion - a rare but potentially fatal complication of blood transfusion occurring in approximately 1 in 34,000 transfusions. It is the result of an acute intravascular hemolysis of transfused red cells. Transfusion of blood group A into blood group O is associated with particularly severe reaction. The reaction often occurs during the first few mls of infusion and may be characterized by dyspnoea, chest and back pain, fever, hypotension, haemoglobinaemia and haemoglobinuria. Major complications such as acute renal failure or disseminated intravascular coagulation (DIC) may occur and are associated with a high mortality. 2. in pregnancy - ABO system incompatibility may cause a mild form of haemolytic disease of the newborn. For example, if the mother is blood group O and the child is blood group A, then there is a marked increase in the titre of anti-A haemolysins, but these drop back to normal levels after the pregnancy. Thus in this type of incompatibility, unlike in Rhesus disease, there is no increased risk for the next pregnancy. If hemolytic disease of the newborn is suspected, the following investigations should be carried out:

- full blood count, with attention to haemoglobin, white cells and reticulocytes,
- infant blood group and Coombs test.

The principle of Coombs' test is the following: addition of the rabbit anti-human IgG to the patient's blood results into the aggregation of the patient's red cells if these red cells are coated with autoreactive IgG. If antisera specific for complement components is added to the patient's blood the presence of complement on the red cells may be demonstrated. A positive Coombs' test is found in cases of autoimmune haemolysis due to the presence of IgG, complement or both, on the surface of the patient's red cells.

Rhesus disease. The D antigen of the Rh system is the most immunogenic (when compared to C and E antigen of the Rh system). Persons who express this antigen are designated as Rh positive. Unlike serum antibodies for the ABO blood types, which

develop spontaneously after birth, Rh antibodies develop after exposure to one or more Rh antigens. More than 80% of Rh-negative persons develop such Abs if they exposed to Rh-positive blood. Because it takes several weeks to produce Abs, a reaction may be delayed and usually is mild. If transfusions of Rh-positive blood are given to a person who has become sensitized, the person may have a severe, immediate reaction.

Rhesus disease can be also a cause of haemolysis manifest in the first 24 hours of life due to rhesus incompatibility between mother and baby. It is the result of a mother being rhesus negative and having antibodies produced towards a rhesus positive baby. It occurs after the mother has been sensitized by either a mismatched blood transfusion, or from fetal blood entering her circulation during miscarriage, abortion, placental bleeding, amniocentesis. Most commonly it occurs at the end of a previous pregnancy during labor and delivery.

In all these instances the sensitizing blood has been rhesus positive. The mother reacts to fetal blood by producing antibodies of anti-Rh D type, which cross the placenta during pregnancy and cause haemolysis of the fetal red cells. Sensitization is more likely if the mother and fetus are ABO compatible, as this ensures that fetal cells persist in the maternal circulation for a more potent immune reaction to be stimulated.

The severity of the condition caused by Rhesus incompatibility varies from the baby born with mild jaundice and anaemia to the development of hydrops fetalis in utero. The latter is usually fatal.

Keep in mind that Rhesus D antigen is the most important and its absence is used to categorize Rhesus negative. However antibodies to other Rhesus antigens may develop (Rh C, Rh E), and can cause Rhesus immunization.

Prevention of Rhesus disease. It is important to identify at risk pregnancies (Rhesus (Rh) negative women).

- anti-rhesus (anti-D) immunoglobulin should be given after delivery to all Rh-negative women where the baby's blood group cannot be determined (e.g. if macerated stillbirth). Also anti-D should be given to Rh-negative mothers following the birth of a Rh-positive infant, immediately or within 72 hours antenatal prophylaxis with anti-D: routine anti-D prophylaxis is offered to all non-

sensitized pregnant women who are RhD negative (routine prophylaxis at 28 and 34 weeks)

- anti-D has the effect of mopping up any rhesus positive cells from the infant in the maternal circulation, and preventing the mother from mounting an immune response. In this way, future pregnancies are protected.

Autoimmune hemolytic anemia. Antibodies are formed against red cell antigens resulting in premature destruction of cells. Autoimmune hemolytic anemia may be due to:

- warm reactive IgG autoantibodies,
- cold reactive IgM autoantibodies /cold haemagglutin disease,
- drug provoked hemolytic anemia,

- complement activating IgG of paroxysmal cold hemoglobinuria.

The combination of autoimmune hemolytic anemia and thrombocytopenia is called Evan's syndrome.

Cold reactive autoimmune hemolytic anemia. Cold hemagglutin disease is a reactive autoimmune hemolytic associated with IgM antibodies to red blood cells. These antibodies react best at low temperature. The majority of cases are idiopathic and present in patients over the age of 60 years. Secondary causes of cold hemagglutinin disease include:

- non-Hodgkin's lymphoma,
- Mycoplasma pneumoniae infection,
- infectious mononucleosis.

The red cells become coated with IgM antibodies in the cooler peripheral circulation. Complement is activated when the blood warms up resulting in intravascular hemolysis. IgM is detectable on the red cells at 4øC. At higher temperatures, IgM detaches from the red cell surface but complement can still be detected. Circulating free cold autoantibodies - cold agglutinins - are also present in the patients serum. A direct Coombs' test may be positive with a reagent containing anti-complement antibodies. Presentation is usually with symptoms of chronic hemolytic anemia i.e. jaundice, anemia, haemoglobinuria.

Warm reactive autoimmune hemolytic anemia is associated with IgG antibodies to red blood cells. These antibodies react best at body temperature. Half of cases are idiopathic. Of the known causes, the most common associations are with lymphoid neoplasms, such as chronic lymphoid leukemia, or autoimmune disease, such as systemic lupus erythematosus. Red cells are opsonized with either antibody alone, or antibody and complement components, and subsequently removed by splenic macrophages. Patients may be of any age but it is most common in people over 30 years of age. Presentation is usually with jaundice and splenomegaly.

The patients red cells are direct Coombs' test positive; they promptly agglutinate when mixed with antiglobulin reagent. In 50% of cases, the red cells have both IgG and C3 fixed on their surfaces; in 40% of cases, IgG only; and in 10%, complement only. Treatment, when required, is with steroids, initially at a high dose which is then tapered off as the response allows. In some patients, splenectomy may be required. Its benefits must, as always, be balanced against the increased risk of infection, especially from capsulated organisms such as Streptococcus pneumoniae.

Post-streptococcal glomerulonephritis. Occurs 7 to 14 days after infection with group A beta haemolytic streptococcus, usually after a sore throat or skin infection. In

tropical climates it is more often seen two or three weeks after skin sepsis. A similar condition can be caused by other bacteria and viruses.

As a consequence of trapping immune complexes (formed from streptococcal antigen, antibodies, and complement) in the glomeruli of the kidneys, the glomeruli become inflamed, causing inefficient filtering and excreting function by the kidneys.

Goodpasture's disease (Goodpasture Syndrome) - anti-glomerular basement membrane disease, is a rare but serious condition that can cause severe kidney and

lung damage. It is an autoimmune condition, in which immune system attacks a target within the body. In Goodpasture's disease the target is the glomeruli and the alveoli. Treatment of type II reactions involves anti-inflammatory and immunosuppressive agents.

Type III hypersensitivity

This type is also known as immune complex hypersensitivity. It can arise with soluble antigens and is mediated by soluble immune complexes. The reaction may be general (e.g., serum sickness) or may involve individual organs including skin (e.g., systemic lupus erythematosus, Arthus reaction), kidneys (e.g., lupus nephritis), lungs (e.g., allergic extrinsic alveolitis, aspergillosis), blood vessels (e.g., polyarteritis), joints (e.g., rheumatoid arthritis) or other organs. This reaction may be the pathogenic mechanism of diseases caused by many microorganisms.

The pathology is caused by the deposition of antigen/antibody aggregates or immune complexes (IC) at certain tissue sites. Immune complexes are generated in all antibody responses but their pathogenic potential is determined, in particular, by the size and the amount, as well as affininty of responding antibody. Large aggregates fix complement and are readily cleared from circulation by the mononuclear phagocytic system. The small complexes that are formed in an antigen excess, however, tend to deposit in blood vessel walls (Fig.10).

- IC can induce inflammation by binding and activation of complement. Upon aggregation in the vessel wall they activate the serine proteases of the complement cascade through the classical route, involving C1q. The complement components C3a and C5a will recruit leukocytes through chemotactic properties. In addition to chemotaxis, the complement cascade can also exert a direct cytotoxic effect. Complement components C5b-9 the so-called membrane attack complex (MAC) is formed on the surface of the cell leading to irreversible injury. Complement as an opsonizing factor plays an important role in phagocytosis and removal of immunoglobulin deposition.

- IC-mediated injury can develop through leukocyte adhesion after binding of antibodies to Fc-receptors. These receptors can be found on phagocytes, natural killer cells and platelets. Chemotaxis of leukocytes and platelets may occur upon complement activation. Activation of endothelial cells is important in the induction of the inflammatory reaction. Cytokines induce endothelial cells to an increased production and expression of leukocyte adhesion molecules by which leukocytes and platelets can adhere and infiltrate through specific receptor-ligand interactions. Degranulation of platelets and leukocytes leads to the release of proteases and oxygen radicals, which are lytic to cell membranes and matrix components.

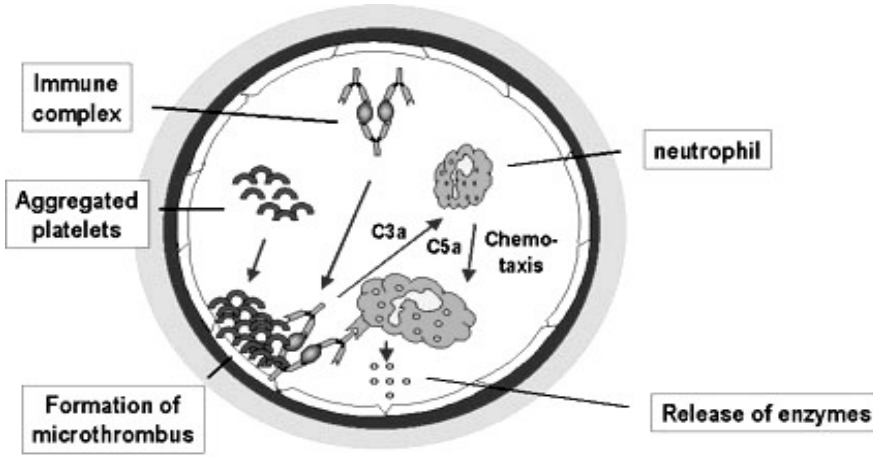


Figure 10. Mechanisms of tissue damage in type III hypersensitivity reaction

A local type III hypersensitivity reaction can be triggered in the skin of sensitized individuals who possess IgG antibodies against the sensitized antigen. When the Ag is injected into the skin, circulating IgG that has diffused into the tissues forms immune complexes locally. The immune complexes find Fc receptors on mast cells and other leukocytes, which creates a local inflammatory response with increase vascular permeability. The enhanced vascular permeability allows fluid and cells, especially polymorphonuclear leukocytes, to enter the site from the local vessels. This reaction is called as Arthus reaction. The immune complexes also activate complement, releasing C5a, which contributes to the inflammatory reaction by ligating C5a receptors on leukocytes. This causes their activation and chemotactic attraction to the site of inflammation.

Another example of the local type III hypersensitivity reaction is an allergic extrinsic alveolitis, which reflect the hypersensitivity reaction within the lungs to an inhaled organic dust. Implicated antigens include avian antigens, fungi and fungal spores, bacterial antigens, and small-molecular-weight chemicals.

Some inhaled allergens provoke IgG rather than IgE antibody responses, perhaps because they are present at relatively high levels in inhaled air. When the person is reexposed to high doses of such inhaled allergen, immune complexes form in the alveolar wall of the lung. This leads to the accumulation of fluid, protein, and cells in the alveolar wall, slowing blood-gas interchange and compromising lung function. This type of reaction occurs in farming, where there is repeated re-exposure to hay dust or mold spores (e.g. thermophilic actinomycetes). The disease that results is therefore called farmer's lung.

Next example of the local type III hypersensitivity is associated with the situation of poorly catabolized antigens. When adaptive immune response fails to clear the

infectious agent, for example in subacute bacterial endocarditis or chronic viral hepatitis, the multiplying bacteria or viruses are continuously generating new antigens. Persistent antibody response that fails to eliminate the organism leads to the formation of immune complexes with the following tissue injury.

A systemic type III hypersensitivity reaction, known as serum sickness, can result form the injection of large quantities of a poorly catabolized foreign antigen. This illness was named because it can follow the administration of therapeutic horse antiserum. In the preantibiotic area, antiserum made by immunizing horses was often used to treat pneumococcal pneumonia; the specific anti-pneumococcal antibodies in the horse serum would help the patient to clear the infection. In much the same way, antivenin (serum from horses immunized with snake venoms) is still used today as a source of neutralizing Abs to treat people suffering from the bites of poisonous snakes.

Serum sickness occurs 7-10 days after the injection of the horse serum, an interval that corresponds the time required to mount a primary immune response that switches from IgM to IgG antibody against antigens in horse serum.

Therefore, the onset of serum sickness coincides with the Abs development. These Abs form immune complexes with the circulating foreign proteins. The complexes are deposited in small vessels and activate complement and phagocytes, inducing fever, chills and the symptoms of arthritis and vasculitis. Urticaria is a prominent feature of the rash, implying a role for histamine derived from mast-cell degranulation. In this case mast cell degranulation is triggered by the ligation of cell-surface FcγRIII by IgG-containing immune complexes.

Serum sickness is usually a self-limiting disease as the formation of immune complex causes clearance of the foreign antigen. Serum sickness after a second dose of antigen follows the kinetics of a secondary antibody response and the onset of the disease occurs typically within a day or two. Serum sickness nowadays seen after the use of anti-lymphocyte globulin, employed as an immunosuppressive agent in transplant recipients, and also, rarely after the administration of streptokinase, a bacterial enzyme that is used as a thrombolytic agent to treat patients with a myocardial infraction or heart attack.

The affinity of antibody and size of immune complexes are important in determining the tissue involved. Diagnosis involves examination of tissue biopsies for deposits of immunoglobulin and complement by immunofluorescence microscopy. The immunofluorescent staining in type III hypersensitivity is granular (as opposed to linear in type II such as seen in Goodpasture's syndrome). The presence of immune complexes in serum and depletion in the level of complement are also diagnostic. Treatment includes anti-inflammatory agents.

Type IV hypersensitivity

Cell mediated or delayed type hypersensitivity (DTH). Unlike the immediate hypersensitivity reactions (types I-III), which are mediated by antibodies, delayed-type hypersensitivity or type IV hypersensitivity reactions are mediated by antigen-specific

effector T-cells. These reactions take some time to develop. The process develops over more than 12 hours and may take 2-3 days to evolve. The reaction is antigen specific and causes erythema and induration at the site of antigen injection or absorbtion. Phagocytic cell activation and inflammation induced by DTH can cause tissue injury.

Basic events in this form of hypersensitivity are:

- Upon antigen recognition, Ag-presenting cells (APCs) process the antigen and present it to local memory T cells, whether they are CD4+ or CD8+.
- These T cells in concert with activated APC secrete numerous cytokines that elicit an influx of macrophages, monocyte, and lymphocytes at the site of Ag exposure. Mononuclear cells produce inflammatory cytokines including tumor necrosis factor-α (TNF-α), IL-17, and IFN-γ.
- Leukocytes migrate through the post capillary venules, venular endothelium is recruiting the cells to the local site.
- The endothelial cells secrete vasodilators such as prostacyclin. The vasodilatation caused by the prostacyclin optimizes delivery of immune cells to the site of challenge.
- The endothelial cells undergo changes as a result of TNF and IFN acting in concert. The endothelial cells remodel the basement membrane and allow the extravasation of plasma macromolecules, especially fibrinogen.
- At the onset of the reaction, vasopermeability is increased by serotonin and histamine, and adhesion molecules are up-regulated in the vascular endothelium, so that additional cellular components migrate into the local site of Ag presentation.
- Endothelial cells are also capable of secreting chemokines which attract various types of cells into the site. IL-8 and MCP-1, both members of the chemokine family, have been shown to be produced by endothelial cells and are capable of increasing the mobility of adherent leukocytes from peripheral blood.
- Mechanisms of damage in delayed hypersensitivity include T lymphocytes and monocytes and/or macrophages.
- Cytotoxic T cells (CD8) cause direct damage.
- Helper T (Th1, CD4) cells secrete cytokines which activate cytotoxic T cells and recruit and activate monocytes and macrophages, which cause the bulk of the damage.
- Major lymphokines involved in delayed hypersensitivity reaction include monocyte chemotactic protein (MCP-1), interleukin-2, interferon-gamma, TNF alpha/beta, GM-CSF.

Type IV hypersensitivity reactions can be grouped into syndromes, according to the route by which antigen passes into the body: 1) In delayed-type hypersensitivity the antigen is injected into the skin. 2) In contact hypersensitivity it is absorbed into the skin. 3) In gluten-sensitive enteropathy it is absorbed by the gut (Table 4).

Delayed-type hypersensitivity. The prototype of this reaction is a tuberculin test used to determine whether an individual has previously been infected with Micobacterium tuberculosis. Small amounts of tuberculin - a complex mixture of peptides and carbohydrates derived from M. tuberculosis – are injected intradermally. In individuals who have previously been exposed to the bacterium, either by infection with pathogen or by immunization with BCG-an attenuated form of M. tuberculosis, a local T-cell-mediated inflammatory reaction evolves over 24-72 hours.

The response is mediated by Th1 cells, which enter the site of antigen injection, recognize complexes of peptide MHC class II molecules on antigen-presenting cells (APCs), and release inflammatory cytokines, such as INF-γ and TNF-β. The cytokines stimulate expression of adhesion molecules on endothelium and increase local blood vessel permeability, allowing plasma and accessory cells to enter the site; this causes a visible swelling. Macrophages are predominant cells in cell infiltrate. Each of these phases takes several hours and so the fully developed response appears only 24-48 hours after challenge.

Table 4. Type IV hypersensitivity syndromes

Syndrome	Antigen	Consequence
Delayed-type hypersensitivity	Proteins: Insect venom, Mycobacterial proteins (tuberculin, lepromin)	Local skin swelling: erythema, induration cellular infiltrate dermatitis
Contact hypersensitivity	Haptens, small metal ions: Nickel, Chromate	Local epidermal reaction: erythema, cellular infiltrate, vesicles, intraepidermal abscesses
Gluten-sensitive enteropathy	Gliadin	Villious atrophy in small bowel, malabsorption

Graft versus host disease (GVHD) is a result of cellular immunity and is an example of a DTH response. A rejected allograft has a similar histological appearance to a tuberculin reaction and rejection is mediated by T cells with a clear role for the NK cell. Also similar to the graft vs. host disease form of cell-mediated immunity are some autoimmune diseases, Hashimoto's thyroiditis, polymyositis.

Contact hypersensitivity (CH). The contact-sensitizing agent is a small highly reactive molecule that can easily penetrate intact skin. It binds covalently as a hapten to a variety of endogenous proteins, which are taken up and processed by Largenhans' cells, the majors antigen-presenting cells in the skin. CHS reactions in mice and humans are mediated by Th1/T cytotoxic 1 (Tc1) effector cells.

There are 2 phases of contact hypersensitivity – sensitization and elicitation. During first phase APCs process Ag, migrate to regional lymph nodes, where they activate T cells, with the consequent production of memory T cells, which end up in dermis. In the second phase further exposure to the sensitizing chemical leads to

antigen presentation to memory T cells in the dermis, with release of T-cell cytokines, such as INF-γ and IL-17. These stimulates the keratinocytes of the epidermis to release cytokines , such as IL-1, IL-6, TNF-α, GM-CSF and chemokines. These cytokines attract monocytes and induce their maturation into tissue macrophages, which contributes to the inflammatory responses.

Some chemicals can cross the cell membranes and modify intracellular proteins. These modified proteins generate modified peptides with the cytosol, which are translocated into the endoplasmic reticulum and are delivered to the cell surface by MHC class I molecules. These are recognized by CD8 T cells, which can cause damage either by killing the eliciting cells or by secreting cytokines such as INF-γ.

Diagnostic tests in vivo include delayed cutaneous reaction (e.g. Montoux test) and patch test (for contact dermatitis). In vitro tests for delayed hypersensitivity include mitogenic response, lympho-cytotoxicity and IL-2 production.

Corticosteroids and other immunosuppressive agents are used in treatment.

IMMUNODEFICIENCIES

Classification

Immunodeficiency can be defined as an abnormality in one or more branches of the immune system that renders a person susceptible to diseases normally prevented by an intact immune system.

5 types of immunodeficiences are divided into 2 subgroups: primary and secondary. Primary immunodeficiency disorders are congenital or inherited abnormalities. Inherited immunodeficiency diseases are caused by recessive gene defects. Before the advent of highly effective antibiotic therapy, it is likely that individuals with inherited immune defects died in infancy or early childhood because of their susceptibility to particular classes of pathogen.

The first immunodeficiency disease was described in 1952 (Bruton's disease). As most of the gene defects that cause these immunodeficiencies are recessive, for this reason, many of the known immunodeficiencies are caused by mutations in genes on the X chromosome. Recessive defects cause disease only when both chromosomes are defective. However, as males have only one X chromosome, all males inheriting X chromosome, carrying a defective gene, will manifest disease whereas female carriers, having two X chromosomes, are perfectly healthy.

1. Humoral (B-Cell) Immunodeficiency

Primary

Transient hypogammaglobulinemia of infancy

X-linked hypogammaglobulinemia

Common variable immunodeficiency

Selective deficiency of IgG, IgA, IgM

Secondary. Increased loss of immunoglobulins (nephrotic syndrome).

Disorders of B-cell function impair the ability to produce antibodies and defend against microorganisms and toxins that circulate in body fluids or enter the body through

the mucosal surface of the respiratory or gastrointestinal tract. Persons with primary B-cell immunodeficiency are particularly prone to pyogenic infections due to encapsulated organisms.

2. Cellular (T-Cell) Immunodeficiency

Primary

Congenital thymic aplasia (Di George syndrome)

X-linked hyper IgM syndrome

Secondary. Malignant disease (Hodgkin's disease and others), transient suppression of T-cell production and function due to an acute viral infection (e.g. measles, AIDS, etc.).

Disorders of T-cell function impair the ability to orchestrate the immune response ($CD4^+$ helper T cells) and to protect against fungal, protozoan, viral, and intracellular bacterial infections ($CD8^+$ cytotoxic T cells). T cells also play an important role in surveillance against oncogenic viruses and tumor; hence, persons with impaired T-cell function are at increased risk for certain types of cancers.

3. Combined B-Cell and T-Cell Immunodeficiency

Primary

Severe combined immunodeficiency (autosomal or x-linked recessive)

Wiskott-Aldrich syndrome (immunodeficiency, thrombocytopenia, and eczema)

Ataxia-telangiectasia

Secondary. Radiation exposure, immunosuppressive vs. cytotoxic drugs, aging.

Combined T-cell and B-cell immunodeficiency states affect all aspects of immune function. Severe combined immunodeficiency represents a life-threatening absence of immune function that requires bone marrow or stem cell transplantation for survival.

4. Complement Disorders

Primary

Angioneurotic edema -complement 1(C1) inactivator deficiency

Selective deficiency in complement components

Secondary. Acquired disorders that involve complement utilization

5. Phagocytic Dysfunction

Primary

Chronic granulomatous disease

Glucose-6-phosphate dehydrogenase deficiency

Chediak-Higashi syndrome

GDI 1/CD18 deficiency

Secondary. Drug induced (corticosteroid and immunosuppressive therapy), diabetes mellitus, chronic stress.

Patients with immune deficiency are usually detected clinically by a history of recurrent infection. The type of infection is a guide to which part of the immune system is deficient. Recurrent infection by pyogenic bacteria suggests a defect in antibody, complement, or phagocyte function, reflecting the role of these parts of the immune

system in host defense against such infections. By contrast, a history of recurrent viral infections is more suggestive of a defect in host defense mediated by T lymphocytes.

1. Humoral (B-cell) Immunodeficiencies

Disorders of B-cell function impair the ability to produce antibodies and defend against microorganisms and toxins circulating in body fluids (IgM and IgG) or enter through the mucosal surface of the respiratory or gastrointestinal tract (IgA).

Persons with primary B-cell immunodeficiency are particularly prone to pyogenic infections due to encapsulated organisms, including those caused by Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, and gram-negative organisms such as Pseudomonas species.

Humoral immunity usually is not as important in defending against intracellular bacteria (mycobacteria), fungi, and protozoa. Viral infections, with the exception of the enteroviruses that cause gastrointestinal infections, rely on cell-mediated immunity and usually are handled normally. Pyogenic, or pus-forming, bacteria have polysaccharide capsules that are not directly recognized by the receptors on macrophages and neutrophils that stimulate phagocytosis. They therefore escape immediate elimination by the innate immune response and are successful extracellular pathogens. Normallly individuals can clear infections by such bacteria because antibody (IgG) and complement (C3b) opsonize the bacteria, making it possible for phagocytes to ingest and destroy them. The principal effect of deficiencies in antibody production is therefore a failure to control this class of bacterial infection. In addition, susceptibility to some viral infections, most notably those caused of enteroviruses, is also increased, because of the importance of antibodies in neutralizing viruses that enter the body through the gut.

Primary humoral immunodeficiency disorders are genetic disorders of the B-lymphocytes. They are the most frequent type of primary immunodeficiencies, accounting for 70% of all primary immunodeficiency disorders.

People with pure B-cell defects resist many pathogens successfully. However, effective host defense against a subset of extracellular pyogenic bacteria fails. These infections can be suppressed with antibiotics and periodic infusions of human immunoglobulin collected from a large numbers of donors. As there are antibodies against many pathogens in this pooled immunoglobulin, it serves as a fairly successful shield against infection.

Transient hypogammaglobulinemia of infancy. During the first months of life, infants are protected from infection by IgG that have been transferred from the maternal circulation during fetal life. The level of maternal IgG gradually declines over a period of 6 months. Between the ages of 1 and 2 years, the child's antibody production reaches adult levels. The total number of circulating B cells is normal but communication between B and T cells that leads to clonal proliferation of antibody-producing plasma cells is reduced. A delay of B cell maturation results in the decrease of serum IgG levels (< 2 standard deviations (SDs) from age-adjusted reference range levels in infants (increase to the reference range by age 2-6 years) (IgM and IgA levels may be

normal). The responses to protein immunizations are typically normal, but the responses to polysaccharide and conjugated polysaccharide antigens (S. pneumoniae) are typically decreased. The result – recurring an upper respiratory tract and middle ear infections. This condition usually resolves by 2 to 6 years of age.

X-linked hypogammaglobulinemia (XLA, Bruton's disease). Described by Bruton in 1952. (An 8-year-old boy presented with frequent pyogenic infections, repeated episodes of sepsis with the same serotypes of Pneumococcus, and multiple episodes of mumps due to the absence of Abs against the pathogens indicated above).

The central defect is a genetic mutation in protein tyrosine kinase named as Btk (Bruton's tyrosine kinase) that blocks proliferation and differentiation of pre-B cells, creating an absence of mature (CD19) circulating B cells and plasma cells. Patients are susceptible to meningitis and recurrent otitis media and to sinus and pulmonary infections induced by an encapsulated pathogens: S. pneumoniae, H. influenzae type b, S. aureus, and Neisseria meningitides.

Diagnosis: 1) history of recurrent infections, mostly in the respiratory tract, through childhood; 2) absence of CD19/CD20 usually easily confirms the diagnosis of XLA in a male (flow cytometry); 3) low or undetectable levels of all serum immunoglobulins (quantitative nephelometry); 4) genetic blood test to confirm the specific Btk mutations.

Treatment: 1) early initiation of therapy is important to ensure the positive outcome; 2) i.v. infusions of Ig (pooled human IgG Abs) every 3–4 weeks, for life (passive immunity) (IgG blood count should exceed 800 mg/kg); 3) prophylactic antibiotics (local treatment -drops, lotions are preferred); 4) avoid the vaccinations with live virus (e.g. oral polio vaccine).

Common variable immunodeficiency.(CVID). Is a group of approx. 150 primary immunodeficiencies (for the majority cases, genetic defects are still unknown). Central defect – the terminal differentiation of mature B cells into the plasma cells is blocked. Clinical manifestations: recurring infections involving the ears, eyes, sinuses, nose, bronchi, lungs, skin, GI tract, joints, bones, CNS, parotid glands, etc. Respond to antibiotics but recur upon discontinuation of the medications. Bronchiectasis can occur from severe and recurrent lung infections.

Diagnosis (diagnosis of exclusion): 1) Symptoms are similar to that of XLA, but the onset of symptoms occurs much later (between the ages of 15 and 35 years); 2) males and females are equally affected; 3) markedly reduced serum immunoglobulin levels (IgG, IgA, half of patients also IgM), accomplished with normal numbers of circulating B lymphocytes (FACs); 3) poor titer response to vaccination with polysaccharide and protein antigens (e.g. pneumococci, tetanus, and diphtheria). 4) exclude loss of the proteins from the kidneys or reduced antibody production secondary to chronic lymphocytic leukemia (CLL) or multiple myeloma;

Treatment - Immunoglobulin therapy (i.v., s.c., i.m).

Selective IgA immunodeficiency. (the most common inherited form of Ig deficiency (1:800). IgA is present in secretions as two antibody molecules attached by a component called the J chain ("J" for "joining"). Most people with low serum IgA have no apparent illness. Some of them (1/3) predispose to the respiratory and gastrointestinal infections. Increased allergic manifestations (asthma, autoimmune disorders - RA, SLE) is also observed.

Diagnosis: 1)chronic or recurrent infections, allergies, autoimmune diseases, chronic diarrhea; 2) moderate to marked reduction of IgA in the blood serum and

secretions (IgG and IgM levels are normal in most cases)(might be IgG2/IgG4 subclasses deficiency).

Treatment - should be directed toward the particular problem (e.g., chronic and recurrent infections – antibiotics). For patients with IgA and IgG2 deficiency who do not respond adequately to antibiotics, the use of replacement gamma globulin may be helpful. An increased risk of anaphylactic reactions when they receive blood products (including i.v. IgG) that contains some of IgA (due to anti-IgA Abs – IgG).

Selective IgG immunodeficiency. (IgG subclass deficiency) The IgG is composed of 4 different subtypes of IgG molecules (IgG1-4) IgG1 (60-70%), IgG2 (20-30%), IgG3 (5-8), IgG4 (1-3%). Therefore, selective IgG subclass deficiency is defined as an absence or very low levels of one or two IgG subclasses (other Ig levels are normal). Abs directed against protein Ags belong to the IgG1 and IgG3, whereas Abs directed against carbohydrate and polysaccharide Ags are predominantly IgG2.

Distribution: selective IgG1 deficiency is very rare, IgG2 is more frequent among children, IgG3 – in adults. IgG4 most often occurs in association of IgG2 deficiency.

Clinical manifestations: persons who are deficient in IgG2 subclass Abs can be at greater risk for development of sinusitis, otitis media, and pneumonia caused by polysaccharide-encapsulated microorganisms (S. pneumoniae, H. influenzae type b, and N. meningitidis.)

Diagnosis: 1) measurement of IgG subclasses is required along with measurement of serum IgG, IgA, and IgM; 2) since the concentrations of IgG subclasses vary up or down over time the normal values are usually“defined”as those values between two standard deviations below and above the average for that person’s age; 3) take into account the clinical status of the patient as well as the ability to produce specific antibodies in response to childhood vaccines (IgG2 subclass deficiency - are unable to produce Abs when immunized with unconjugated polysaccharide vaccines against Streptococcus pneumoniae or Haemophilus influenzae, but usually make normal amounts of Abs to the protein vaccines such as diphtheria and tetanus toxoids in the routine DPT immunizations; 4) normal numbers of B and T-lymphocytes and T-lymphocytes function normally when tested.

Treatment: 1) antibiotics; 2) for patients whose infections cannot be readily controlled with antibiotics, or have abnormal antibody responses, immunoglobulin replacement therapy may be considered

Secondary (B-cell) humoral Immunodeficiency disorders can develop as a consequence of selective loss of immunoglobulins through the gastrointestinal or genitourinary tracts. For example, nephrotic syndrome – due to the abnormal glomerular filtration, serum IgA and IgG are lost with the urine, IgM is not filtered into the urine because of its larger molecular size, and serum level remains normal.

Cell-mediated (T-cell) Immunodeficiencies

Unlike the B-cell lineage or immunodeficiency, in which a well-defined series of differentiation steps ultimately leads to the production of immunoglobulins, mature T lymphocytes are composed of distinct subpopulations whose immunologic assignments are diverse. T cells can be functionally divided into two subtypes ($CD4^+$ helper and $CD8^+$ cytotoxic T cells). Collectively, T lymphocytes protect against fungal, protozoan, viral, and intracellular bacterial infections; control malignant cell proliferation; and are responsible for coordinating the overall immune response.

Primary Cell-Mediated Immunodeficiency Disorders - in general, persons with cell-mediated immunodeficiency disorders have infections or other clinical problems that are more severe than antibody disorders. Children with defects in this branch of the immune response rarely survive beyond infancy or childhood. However, exceptions are being recognized as newer T-cell defects, such as the X-linked hyper-IgM syndrome, are identified. In this case, T-cell defects can result in low antibody levels.

Secondary deficiencies of T-cell function are more common than primary deficiencies. In the case of viruses, direct infection of specific T-lymphocyte subpopulations (e.g., helper cells) by lymphotropic viruses such as the human immunodeficiency virus (HIV) and human herpesvirus type 6 can lead to loss of the cellular function and selective T-lymphocyte subtype depletion with a concomitant loss of immunologic function associated with that subtype.

Persons with neoplastic disorders can have impaired T-cell function based on unregulated multiplication or dysfunction of one particular subclone of T cells.

The outward expression of this may be an increased susceptibility to infections caused by normally harmless pathogens (i.e., opportunistic infections) or failure to generate delayed-type hypersensitivity reactions (i.e., anergy). Persons with anergy have a diminished or absent reaction to a battery of skin test antigens, including Candida and the tuberculin test, even when infected with Mycobacterium tuberculosis. In persons with anergy, a negative skin test result for tuberculosis can mean a true lack of exposure to tuberculosis or indicate the person's inability to mount an appropriate T-cell response.

Congenital thymic aplasia (Di George syndrome) The central defect: embryonic defect resulting in the partial or complete failure of development of the thymus and

parathyroid glands. This results from microdeletion of 30-50 genes on chromosome 22 (22q11.2) with diminished expression of multiple genes acting on common cellular processes during the development of the brain, heart, face, and limbs, and, subsequently, in the adolescent and adult brain.

Diagnosis: 1) facial features (eg., underdeveloped chin, eyes with heavy eyelids etc.); 2) Parathyroid gland abnormalities: low blood calcium on a routine blood test, or the infant may have convulsions as a result of the low calcium; 3) signs of heart failure (low oxygen content of the arterial blood - cyanotic); 4) signs of infection (underdevelopment of the thymus gland and low T-lymphocyte levels).

Tteatment: 1) low calcium levels and hypoparathyroidism may involve calcium supplementation and replacement of the missing parathyroid hormone; 2) corrective surgery to improve the function of the heart; 3) in most cases the small amount of thymus that is present provides adequate T-lymphocyte function - in severe cases, thymus transplant or bone transplantation may be performed; 4) may require immunoglobulin replacement therapy (rarely, in the when T-lymphocyte defect is significant enough to cause the B-lymphocytes to fail to make sufficient Abs).

X-linked hyper-IgM syndrome. An inability to switch Ab production from IgM to IgG, IgA, and IgE. As a result, patients have decreased levels of IgG and IgA and normal or elevated levels of IgM. Molecular mechanism: inability of T cells to "instruct" B cells to switch their Ab production from IgM to IgG and IgA due to a defect or deficiency of CD40L expression of activated T-lymphocytes. This produces susceptibility to infection with extracellular pathogens. Affected boys have recurrent pyogenic infections, including otitis media, sinusitis, tonsillitis, and pneumonia.

Diagnosis: 1) the decreased levels of IgG and IgA and normal or elevated levels of IgM in serum; 2) failure to express CD40 ligand on activated T-cells is a characteristic finding; 3) neutropenia; 4) identification of a mutation affecting the CD40 ligand gene.

Treatment: 1) immunoglobulin replacement therapy (every 3 to 4 weeks) is effective in decreasing the number of infections; replacement of the missing IgG often results in the normalization of IgM; 2) granulocyte colony stimulating factor (G-CSF) therapy for the patients with persistent neutropenia; 3) prophylactic or preventative treatment for Pneumocystis cariniii pneumonia (Bactrim); 4) should not receive live virus vaccines; 5) bone marrow transplantation or cord blood stem cell transplantation.

2. Combined B-cell and T-cell immunodeficiencies

Disorders that affect both B and T lymphocytes, with resultant defects in both humoral and cell-mediated immunity, fall under the broad classification of combined immunodeficiency syndrome (CIDS). A single mutation in any one of the many genes that influence lymphocyte development or response, including lymphocyte receptors, cytokines, or major histocompatibility antigens, can lead to combined immunodeficiency. Regardless of the affected gene, the net result is a disruption in the normal communication system of B and T lymphocytes and deregulation of the immune response. The spectrum of disease resulting from

combined immunodeficiency disorders ranges from mild to severe to ultimately fatal forms.

The most pronounced form of the combined immunodeficiencies often is referred to as severe combined immunodeficiency syndrome (SCIDS). A disease course resembles AIDS, usually lead to death by the age of 2 years. Combined immunodeficiency syndrome (CIDS) is distinguished from SCIDS by the presence of low, but not absent, T-cell function. Although antibody-forming capacity is impaired in most cases, it is not absent. Like SCIDS, however, CIDS is a syndrome of diverse genetic causes and is often associated with other disorders such as ataxia-telangiectasis and Wiskott-Aldrich syndrome (eczema and thrombocytopenia).

Children with CIDS are prone to develop recurrent pulmonary infections, failure to thrive, oral and cutaneous candidiasis, chronic diarrhea, recurrent skin infections, gram-negative sepsis, and urinary tract infections. Although they usually survive longer than children with SCIDS, they fail to thrive and often die early in life.

Severe combined immunodeficiency (SCID). The defining characteristic of SCID: absence of T cells and, as a result, lack of B cell function as well (in some cases, a lack of natural killer (NK) cells). X-linked inheritance or X-SCID form only males and accounts for approximately 45% of all cases of SCID. Autosomal recessive form of SCID (child inherits 2 defective copies of the same autosomal gene).

X-linked SCID arises due to the mutations in the gene (Xq13) encoding the cytokine receptor common gamma chain (IL2RG) that induce the differentiation and maturation of T lymphocytes. X-SCID is a combined cellular and humoral immunodeficiency resulting from lack of T and natural killer (NK) lymphocytes and nonfunctional B lymphocytes. Clinical manifectations: failure to thrive, oral/diaper candidiasis, absent tonsils and lymph nodes, recurrent infections, infections with opportunistic organisms such as Pneumocystis, and persistence of infections despite conventional treatment.

Diagnosis: 1) Lymphocytes count - the number of T cells is usually very low, B cells are generally present, but nonfunctional and the number of NK cells is low or absent; 2) Typical X-SCID is designated T–B+NK–; 3) Lymphocyte functional tests: a) Antibody responses to vaccines and infectious agents are absent; b) T-cell responses to mitogens are lacking; 4) Igs concentrations: - IgA and IgM levels are low, IgG is generally normal at birth, but declines as maternally transferred IgG disappears by 3 months of age; 5) Thymus - thymic shadow is absent on chest radiogram; 6) Genetic testing - mutations, insertions, deletions in IL2RG (family-specific).

Management: bone marrow transplantation (BMT)-HLA-matched bone marrow from a relative or haploidentical parental bone marrow depleted of mature T cells; 2) immunoglobulin infusions; 3) antibiotics, particularly prophylaxis against Pneumocystis; 4) gene therapy - autologous bone marrow stem/progenitor cells retrovirally transduced with a therapeutic gene (as an option for those who are not candidates for BMT or have failed BMT)

Autosomal recessive SCID may be due to the deficiency in adenosine deaminase (ADA) due to the gene mutation on chromosome 20. Absence of this enzyme leads to: 1) inhibition of ribonucleotide reductase and prevention of DNA synthesis, so the cells are unable to divide; 2) an increase in S-adenosylhomocysteine, since ADA is important in the purine salvage pathway; the substance is toxic to immature lymphocytes, which thus fail to mature.

Diagnosis: 1) evidence of combined immunodeficiency: a) the numbers of T, NK and B cells are very low or absent. Typical ADA is designated as T (-) B (-) NK (-); b) lymphocyte functional tests - proliferative responses to antigens and mitogens are low or absent; c) Igs concentrations - serum Igs are low, no specific antibody response to infections and immunizations; 2) adenosine deaminase (ADA) catalytic activity is < 1% of normal ADA catalytic activity in erythrocytes hemolysates 3) genetic testing - ADA mutations.

Management: 1) treatment of manifestations - infections are treated with specific antibiotic, antifungal, and antiviral agents and administration of intravenous immunoglobulin (i.v. Ig); prophylaxis is provided for Pneumocystis carinii infection); 2) bone marrow/stem cell transplantation (BMT/SCT) from an HLA-identical healthy

sib or close relative (preferential); 3) for the individuals, lacking an HLA-identical related donor alternative therapies - enzyme replacement therapy (ERT) - polyethylene glycol-modified bovine adenosine deaminase (PEG-ADA, Adagen®)

Ataxia-telangiectasia (A-T) (Louis–Bar syndrome). Autosomal recessive disease, result of mutation in the ATM (Ataxia Telangiectasia Mutated) gene (11q22.3). A-T affects:

1) certain areas of the brain, including the cerebellum (difficulties with movement and coordination);

2) impaires the immune systen (a predisposition to infection);

3) impairs the repair of broken DNA (increase risk of cancer and radiosensitivity).

Clinical manifstations: 1) worsening cerebellar ataxia (i.e., poor muscle coordination) and the appearance of telangiectases (i.e., lesions consisting of dilated capillaries and arterioles) on skin and conjunctival surfaces; 2) deficiencies in both cellular and humoral immunity - reduced levels of IgA, IgE, and IgG2, absolute lymphopenia, a decrease of CD4+/CD8+ ratio; 3) an increased susceptibility to recurrent upper and lower respiratory tract infections (particularly those caused by encapsulated bacteria); 4) increased risk for the development of malignancies (ATM-kinase linked to the DNA repair pathways).

3. Complement Disorders

The complement system is an integral part of the innate or nonspecific immune response. The activation of the complement network via the classic or alternative pathways promotes chemotaxis, opsonization, and phagocytosis of invasive pathogens, bacteriolysis, and anaphylactic reactions. Thus, alterations in normal levels of complement or the absence of a particular complement component can lead

to enhanced susceptibility to infectious diseases and immune-mediated disorders such as hemolytic anemia and collagen vascular disorders. Infections associated with complement deficiencies overlaps substantially with that seen in patients with deficiencies in antibody production.

In particular, "early" components C1 (C1q, r, and s) and C4 are important for the elimination of immune complexes and clearance of apoptotic cells. The deficiency of these components may create conditions that allow for the development autoimmunity to self-antigens.

Patients with C1q deficiency have the highest incidence of systemic lupus erythematosus (SLE), an SLE-like syndrome. Early complement deficiencies (C1q, C1r, C1s, C4, and C2) typically do not result in a significant increased susceptibility to infections. In contrast, since C3b fragment acts as the main opsonin, C3b deficiency is usually associated with recurrent infections induced by the encapsulated microorganisms (*Pneumococcus, H. influenzae*).

The terminal complement components (C5-9) mediate an extracellular lysis of the bacteria due to the formation of "membrane attacking complex" (MAC) making the people having such defect a succeptable for recurrent systemic bacterial infections (especially induced by *Neisseria* species *(N. meningitides and N. gonorrhoeae*). C9 deficiency is the most common complement deficiency in Japan occurring in 0.1% of the population.

Diagnosis of complement system deficiency: 1) the evaluation for the classical complement deficiencies should be considered in the patients with recurrent infections with the encapsulated microorganisms; 2) a history of autoimmune disease (SLE) may be seen in early complement deficiencies; 3) recurrent *Neisserial* infections can be a feature of terminal complement deficiencies.

Lab tests: 1) CH50 assay based on the ability of a patients serum to lyse sheep red blood cells coated with antibody. A complete deficiency of any of the classical complement components will yield a very low or undetectable CH50 value; 2) individual Classical Complement Component Levels (if CH50 is low); 3) gene Sequencing (identification of mutations in complement protein genes to confirm the diagnosis).

Management: 1) Appropriate management of autoimmune disease - a key issue for many patients with early complement deficiencies; 2) Vaccination for encapsulated bacteria for the patients suffering from infections with encapsulated organisms. Patients with terminal complement component deficiencies might be considered to receive the meningococcal vaccinations. (Periodic evaluations to confirm the presence of protective specific antibody titers is recommended). 3)Prophylactic antibiotics - may be used as an adjuncive therapy for the patients suffering from frequent infections despite the vaccination procedures.

Hereditary Angioneurotic Edema (HAE) types I and II (C1 esterase inhibitor deficiency) are due to the mutations in the SERPING1.

Type I – an absence of C1 inhibitor (normally prevents activation of a cascade of the proteins); Type II – C1 inhibitor is present but is non-functional. Activation of the

classic complement pathway is uncontrolled, leading to an increased breakdown of C4 and C2 with concomitant release of C2 kinin, bradykinin. Therefore, an edema is induced by an uncontrolled generation of the vasodilators. This causes episodic attacks of localized edema involving face, neck, joints, abdomen, the sites of trauma. Swelling of the gastric mucosa causes nausea, vomiting, and diarrhea, if the trachea or larynx is involved, the episode might be fatal. The attacks usually begin before the age of 2 years and become progressively worse with age. Symptoms can last from 1 to 4 days, and most people with HAE have more than one attack per month.

Diagnosis: abnormally low levels of C1 esterase inhibitor in the blood

Treatment: 1) anti-histamines to prevent the recurrent attacks; 2) i.v. C1 esterase inhibitor (plasma-derived) – Cinryze (ViroPharma), or recombinant - Ruconest (Rhucin); 3) Bradikynin B2 receptor antagonist – Icatibant (Firazyr); 4) Kallikrein inhibitor – Kalbitor (Dyax Corp)

Deficiency of decay-accelerating factor (DAF) and CD59 is a defect in the protein controlling the activation of the complement system and thereby protecting the cellular surfaces (in particular, red blood cells) from the complement activation. This defect results in the paroxysmal nocturnal hemoglobinuria.

Secondary Disorders of the Complement System can be due to: rapid activation and turnover of complement components (immune complex diseases, the lower levels of the complement components as a result of an increased consumption) or reduced synthesis of components (chronic cirrhosis, malnutrition). Functional activity of complement components is normal.

4. Phagocytic Dysfunction

Most of the defects in phagocytic cells affect their ability to kill intracellular and/or extracellular bacteria. Patients have chronic bacterial infections, which in some cases lead to the formation of granulomas.

Chronic granulomatous disease (Bridges–Good syndrome) - group of inherited disorders with the reduced ability of the phagocytic cells to produce oxygen components (also known as a “respiratory burst”). Recurrent infections, along with granulomatous lesions, are due to the persistence of viable microorganisms in the impaired phagocytic cells. These infections usually begin during the first 2 years of life. Bone marrow transplantation (BMT) from a matched donor is recommended. Supportive care includes the use of recombinant interferon-γ and prophylactic antibiotic therapy.

Chediak-Higashi syndrome is due to the mutations in LYST (currently termed CHS1)(1q42-43). This protein is known as a lysosomal trafficking regulator – i.e. fusion of phagosomes with lysosomes is desrupted and intracellular killing of the pathogens is impared.

Syndrome is characterized by: a) hypopigmentation of the skin, eyes and hair (partial albinism); b) prolonged bleeding times (abnormal platelet function); c) recurrent infections (severe immunodeficiency- neutropenia, impaired chemotaxis, delayed phagolysosomal fusion); d) nonmalignant lymphohistiocytic lymphoma-like infiltration of multiple organs (>80% of patients).

Secondary deficiencies of the phagocytic system can be caused by a number of circumstances, such as deficiencies of opsonins (e.g., antibody and complement), which coat the surface of a foreign substance and enhance phagocytosis, and deficiencies of chemotactic factors which promote migration of the phagocytes to the site of infection and stimulate phagocytosis. Deficiencies of either of these factors reduce the overall effectiveness of phagocytes. Drugs that impair or prevent inflammation and T-cell function, such as corticosteroids or cyclosporine, also alter phagocytic response through modulation of cytokines.

To determine the competence of the immune system in patients with possible immunodeficiency, a battery of tests is usually conducted; these focus with increasing precision as the nature of the defect is narrowed down to a single element. The presence of the various cell types in blood is determined by:

- routine hematology (complete blood count…)
- often followed by FACS (flow cytometry) analysis of lymphocyte subsets
- the measurement of serum immunoglobulins
- the phagocytic competence of freshly isolated polymorphonuclear leukocytes and monocytes is tested. (e.g., CGD is
- diagnosed by examining the ability of phagocytes to reduce a yellow dye (i.e., nitroblue tetrazolium) to a blue compound during active respiration)
- the efficiency of the complement system is determined by testing the dilution of serum required for lysis of 50% of antibody-coated RBCs

In general, if such tests reveal a defect in one of these broad compartments of immune function, more specialized testing is then needed to determine the precise nature of the defect. Tests of lymphocyte function are often valuable, starting with ability of polyclonal mitogens to induce T-cell proliferation and B-cell secretion of immunoglobulin in tissue culture.

MECHANISMS OF TUMORIGENESIS

The most fundamental characteristic of the cells is their ability to reproduce themselves. Living cells go through a series of stages known as the cell cycle (Fig. 11). The cells grow, copy their chromosomes, and then divide to make the new cells.

The cell cycle is the ordered series of events required for the faithful duplication of one eukaryotic cell into two genetically identical daughter cells. G1 phase - the cell grows. S phase-the cell makes copies of its chromosomes, each chromosome now

consists of two sister chromatids. G2 phase-the cell checks the duplicated chromosomes and gets ready to divide. M phase-the cell separates the copied chromosomes to form two full sets (mitosis) and the cell divides into two new cells (cytokinesis). The period between cell divisions is known as 'interphase'. Cells that are not dividing leave the cell cycle and stay in G0.

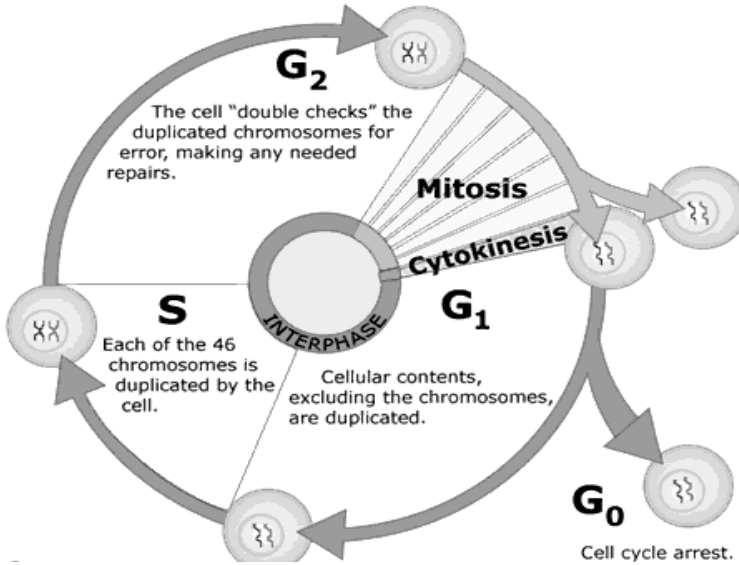


Figure 11. The cell cycle

Both internal and external inputs trigger molecular events that regulate normal progress through the stages of the cell cycle.

Many external factors affect cell division. Both normal and abnormal cell cycles can be triggered by such extrinsic controls. Normal cell growth is controlled by the availability of growth factors, which are hormone-like molecules that bind to specific receptors in the surface membrane of cells. When this happens, the receptor stimulates a signaling cascade inside cells that ultimately tells the cells to divide. PDGF - platelet-derived growth factor, EGF - epidermal growth factor, IL2 and many others initiate cell proliferation; on the other hand TNF, INF – suppress the process. In the nucleus, these normal growth signals trigger other proteins, called transcription factors, that regulate gene expression needed for cell growth. Gene products in this signaling pathway are proto-oncogenes. These genes encode proteins that function as growth factors, growth factor receptors, signal-relaying molecules, and nuclear transcription factors (proteins that bind to genes to start transcription). The Ras proto-oncogene is an

example of a signal-transmitting molecule inside cells. Two examples of proto-oncogene transcription factors are c-Fos and c-Jun.

External signal can be initiated by hormones, for example, the hormone estrogen affects the development of a wide variety of cell types in women. Estrogen exerts its effects on a target cell by binding to a specific receptor protein on the cell's nuclear membrane. By binding to an estrogen receptor, estrogen initiates a cascade of biochemical reactions that lead to changes in the cell-cycle program. Normally, estrogen moves cells out of a resting stage into an active cell cycle.

Intrinsic control of the cell cycle is exerted by cyclin-dependent kinases (CDKs) and checkpoints. Without complete and proper DNA replication, the events of mitosis are not initiated. This control of cell-cycle order is maintained through an intracellular «checkpoint» that monitors the integrity and completion of DNA synthesis before authorizing the initiation of mitosis. Progression through the cell cycle is highly regulated, particularly at the transitions from G1 phase to S phase and from G2 phase to M phase. These two «checkpoints» assess cell-intrinsic signals (for example, the integrity of the genome) and are governed by the cyclin dependent kinases CDK2 and CDK1.

The coordinated transitions between cell cycle stages depend on one family of evolutionarily conserved proteins, called cyclin-dependent kinases. Cyclin-dependent kinases (CDKs) act as oscillating driving forces to direct the progression of the cell cycle. Each CDK consists of two parts, an enzyme known as a kinase and a modifying protein called a cyclin. Kinases are regulatory enzymes that catalyze the addition of phosphate groups to protein substrates. Adding one or more phosphate groups to a substrate protein can change that substrate's ability to do its cellular job. Cyclins, so named because their activity cycles up and down during the cell cycle, restricts the action of their bound kinase to particular substrates. Together, the two integral parts of a CDK target specific cellular proteins for phosphorylation, thereby causing changes in cell-cycle progression. Each CDK, consisting of a particular kinase bound by a particular cyclin, directs a critical transition in the cell cycle. For example, one CDK controls the initiation of DNA synthesis, while another CDK controls the onset of mitosis. Inactivation of the mitotic CDK is necessary for a subsequent cell-cycle transition, when cells exit mitosis and proceed to G_1. CDKs are also the ultimate targets of most cell-cycle checkpoint activity. Thus each of a cell-cycle event occurs at the proper time during each cell cycle, CDK activity itself is tightly controlled by regulating the activity of every cyclin. Each cyclin is active only periodically during the cell cycle, with its peak of activity limited to the period during which it is needed. Regulated transcription of cyclin genes and regulated degradation of cyclin proteins provides this oversight.

For the cell to move from one stage of its life cycle to the next, certain proteins must be activated while others must be inhibited. The induction of apoptosis (programmed cell death) and the cell cycle are intimately related. The molecular cascade of apoptosis is characterized by the activation of caspases (3, 8, 10, effector caspase 9) in a self-amplifying cascade. The cascade of apoptosis is subject to

regulation at several levels. Members of the Bcl-2 family of proteins may be either antiapoptotic (Bcl-2, Bcl-xL, Mcl-1) or proapoptotic (BAD, Bax, Bak).

Tumour suppressor genes are genes involved in the cyclin and apoptotic pathways. Normally tumour suppressors detect breaks or defects in the DNA – if present in low concentrations these proteins will pause the cell cycle and active DNA repair mechanisms. If present in high concentrations, tumour suppressors shut down the cell cycle or cause apoptosis. When these genes are mutated to be dysfunctional then the cell does not undergo either of these events.

G1-S-phase checkpoint responds to various forms of DNA damage, such as single-and double-strand breaks in the DNA or incorporation of unusual nucleotides, and halts the progression of the cell cycle until effective repairs have occurred. Normally, the gene product p53 induces a cell suicide program - apoptosis in cells with damaged DNA. Loss of p53 activity allows cells with damaged DNA to grow and pass DNA mutations to their daughter cells.

4 main types of genes regulating a cell-cycle division:

- Proto-oncogenes (initiate cellular division),
- Tumor suppressor genes (genes halting cell-cycle progression after DNA damage)
- Apoptosis-related genes (genes activating a cellular suicide program, if DNA damage is un-repairable),
- Genes involved in repair of DNA lesions

Tumors represent the form of the abnormal and uncontrolled cell growth occurring upon the carcinogen exposure. The carcinogens are able to initiate the turn of normal cell into a cancer cell. This process is known as a carcinogenesis.

Carcinogenesis is the term for the mechanism by which cancer develops. Broadly, for some cancers there is one particular factor that is associated with the instigation or maintenance of the state of abnormal growth. However, for most cancers, carcinogenesis is thought to be a multi-stage process of disruption to the genes, which control cellular proliferation and differentiation.

Molecular regulation of cellular proliferation and differentiation. As mentioned above, cellular proliferation and differentiation is a balance of elements, which tend to enhance or retard each function. Growth factors have their signal transduced at the nucleus into a complex web of activation of two types of genes:

1. Proto-oncogenes are genes coding for proteins involved in cell cycle progression and growth signalling; when mutated these genes are named as an oncogenes.

Those “normal” cellular genes after being switched on provide an increased activity, thus leading to the overproduction of the proteins stimulating the cellular growth and proliferation.

- they act via tyrosine kinase production, and hence protein phosphorylation, G protein or nuclear protein production

- can be activated by point mutations, translocations of regions of chromosomes, or duplication and hence excessive production of the proto-oncogene
- several oncogenes have been identified in many types of cancers, including Myc, (bcl family) and Ras which is derived from a proto-oncogene by a point mutation.

2. Tumour suppressor genes are the normal cellular genes switching off or suppress cellular proliferation.

- they are involved in regulating apoptosis, cells that have sustained damage to their DNA apoptose under the control of tumor suppressors
- the inactivation or under-expression of such genes could stimulate over-proliferation of cells
- may be disrupted by the same molecular mechanisms as those affecting proto-oncogenes, e.g. point mutation
- an example of tumor-suppressing gene is p53, which is known as a potent cell cycle regulator

The activity of this protein increases after a DNA damage. This protein blocks cell cycle in G1 stage and induces a programmed cell death (apoptosis) in the cells failed to repair DNA damage. The mutation of the gene encoding this protein leads to uncontrolled proliferation of cells containing non-repaired DNA. More than 50% of tumors have mutated and therefore non-functional p53 gene.

Carcinogens

Factors known to induce a cancer (named as carcinogens) are classified into 2 groups – exogenous and endogenous.

- Exogenous carcinogens are the following: chemicals (e.g., polycyclic aromatic amine hydrocarbons, etc), physical factors (ionizing radiation, UV-light exposure), biological factors (e.g. viruses – HPV, HBV, Epstain-Barr virus, HTLV, etc).
- Endogeonus carcinogens are the products of the metabolic pathways (tyrosine - phenyloksylactic acid, triptophane – orthaminophenols).

Carcinogenesis

Carcinogenesis includes 3 stages: initiation, promotion and progression.

Initiation stage is associated with the genetic changes induced by various types of carcinogens, which predispose the normal cells to aquire the malignant phenotype. It represents the interaction between carcinogens and DNA sites, coding the proteins which can be involved in:

- proliferation and differentiation (proto-oncogens, e.g. Ras),
- cell-cycle control (e.g. tumor-supressor proteins - pRb, p53, p21, etc.),
- DNA damage repair and maintenance of genetic stability (ATM, Mre11, FancD2, etc.).

The initiated cell is not a neoplasic cell but has taken its first step towards this state. From a phenotypical perspective, the initiated cell is basically similar to the remaining cells. It undergoes mutations and these induce proliferation but not differentiation. The initiated cells can remain latent for weeks, months or years, or they can grow in an autonomous and clonal fashion. The clonal expansion of initiated cells results from amitogenic process caused by an increase in the number of new cells and apoptosis inhibition, which prevents initiated cells from dying off.

2 types of the interactions between carcinogens and DNA are currently known:

- Genome variant is characterized by the point mutation of pro-oncogene.
- Epigenetic variant is characterized by the de-repression of previously non-active proto-oncogene.

In both cases it leads to the tumor transformation of the cell. After this event the process becomes an irreversible. The main consequence of this step of carcinogenesis is Hayflick's limit loss – the ability of the cells to limited number of mitotic cycles. The cell becomes immortal and can devide indefinite amount of times. Leonard Hayflick discovered that non-stem cells could only divide around 60 times. The limit on a cell's maximum lifespan with respect to its descendants is known as its "Hayflick's limit." The DNA molecule of a typical chromosome contains a linear array of genes (encoding proteins and RNAs) and stretches at the ends of the chromosome- the telomeres. Telomeres are crucial to the life of the cell. They keep the ends of the various chromosomes in the cell from accidentally becoming attached to each other. The ends of the chromosomes get shorter every time the cells divide, so that the telomeres become shortened with each mitosis. Thus, after every round of replication, the chromosomes gets shorter – telomeres in cell culture grow shorter with age, telomeres are shorter in older individuals.

However the cells have a mechanism for extending the length of the telomeres - the cells have an enzyme, which can make the telomeres longer. The telomerase enzyme is a ribozyme - that is, it contains a necessary piece of RNA, which serves as the template for synthesizing the new strand. It has been found that in many cases cancer cells have a mutation such that the telomerase gene is overexpressed, thus allowing the cells to "live forever".

Promotion stage represents the interaction between initiated cell and the various growth factors. The consequence of this interaction is tumor growth.

In general, tumor promotion can be viewed as the clonal expansion of an initiated cell via altered gene expression that gives the cell a selective growth advantage. Tumor promoters cause cells to proliferate but not to terminally differentiate, resulting in proliferation of the cells. The promoter must be present for weeks, months and years in order to be effective and its effectiveness depends from its concentration in the tissues. Promotion is a reversible stage, after a promoter's disappearance a regression of the cellular proliferation can occur, probably by apoptosis.

Unlike initiators, most of the promoters do not bind covalently to DNA and usually do not cause mutations. On the other hand, these promoters may indirectly

damage DNA by oxidation. At first, these occurrences were associated with epigenetic mechanisms, but nowadays it is widely agreed that promotion also involves genetic changes. Thus, the promoter might have an indirect effect - by increasing the frequency of cellular division it encourages the appearance of errors in DNA replication, as well as mutations. (Fig. 12).

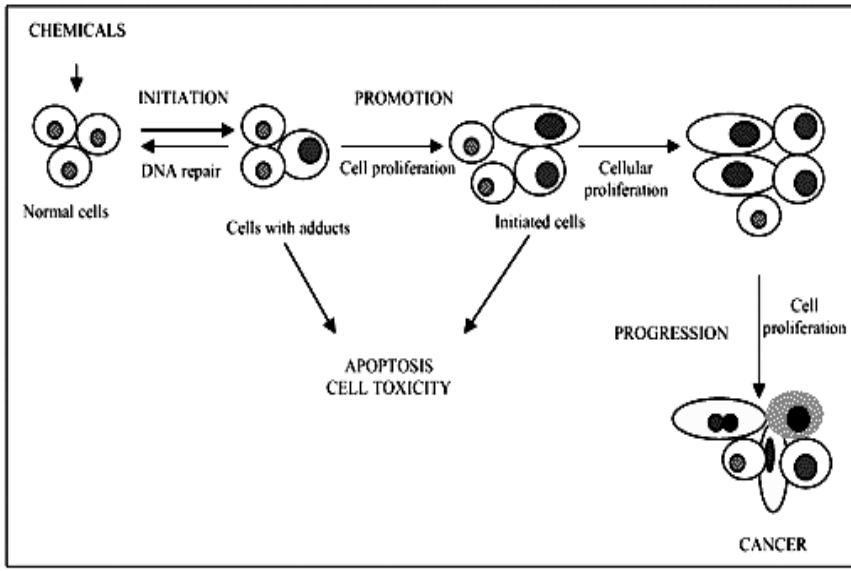


Figure 12. Stages of carcinogenesis

Tumor progression describes the process whereby tumors acquire the ability to grow, invade local tissue and establish distant metastases. Increased genetic instability and karyotypic alterations are hallmarks of progression. Inherited or acquired mutations in genes such as p53 or DNA mismatch repair can increase the rate of mutation in other genes (mutator phenotype) and, therefore, promote the tumor progression.

As a result of the various carcinogens exposure (chemicals, radiation, UV-light, integration of the viral genome), 3 types of events might happen, which might have a potential role in the transforming of normal cells into the tumor cells:

- the conversion of proto-oncogenes to oncogenes
- the inhibition of tumor suppressor genes
- the inhibition of DNA repair genes

First, proto-oncogenes, which encode proteins for cell growth and which are normally tightly regulated, become oncogenes whereby they never stop producing growth related proteins.

Second, genes that would normally suppress these oncogenes get turned off.

Third, genes, which encode the proteins, which fix DNA mutations get turned off stunting the cells ability to regain control.

Basically if any of these three things do not occur then the cell would either remain normal or the cancer cell would form but not survive. The immune system also contributes to destroying cancer cells by recognizing abnormalities on the cancer cells membrane. These various defense mechanisms explain why the rate of cancer in human beings is relatively low compared to world population. Although carcinogen exposure is required for cancer development, the group of factors able to influence this process has been also discovered.

Hereditary factors

Hereditary predisposition is present for a range of cancers. In some cases there is a multifactorial link, as evidenced by the higher rate of lung cancer relative to controls in the non-smoking relatives of patients with this condition. There is no certainty that the relatives will develop lung cancer, but they are at higher relative risk. However, for the some forms of cancer there is a much greater chance to develop this condition associated with the carriage of a given gene defect within a family:

- early onset familial 17q - breast cancer
- familial adenomatous 5q - polyposis
- Li-Fraumeni 17p
- MEN type I 11q
- MEN type II 10
- neurofibromatosis type I 17q
- neurofibromatosis type II 22q
- retinoblastoma 13q
- Wilm's tumour 11p

Certain cancers provide a model for understanding the molecular mechanisms of hereditary susceptibility. A prime example is retinoblastoma where 2 forms of the Rb gene exists on chromosome locus 13q. In the hereditary form, each cell has a normal and abnormal copy of the gene. The normal gene dictates that the cell remains normal until this copy is itself mutated. Consequently, bilateral retinoblastomas develop at an early age as soon as somatic mutation has occurred in one gene in one cell in both eyes. A sporadic form of retinoblastoma occurs in individuals with normal 13q loci. Both genes in each cell must be mutated and because the chance of this occurring is less than the chance of one gene mutating in the hereditary form, sporadic retinoblastoma tends to occur in one eye only at a later age.

The frequency of genetic or inherited forms of cancer is relatively low. A primary cause of cancer is the mutation in the specific gene. If the mutated gene is part of the genetic line, then the cancer can be inherited by succeeding generations. However, if the mutated gene is a somatic or general body cell, as most cancers appear

to be, then the cancer will not be passed to the further generations. The genetic or inherited cancers can be passed along through autosomal dominant, autosomal

recessive, and X-linked transmission. Examples of inherited cancers include familial forms of breast cancer, polyposis of the colon, adenomas of the colon, retinoblastomas (a childhood cancer of the eye), Wilms tumor (a childhood cancer of the kidney), and neurofibromatosis.

The mechanisms involved in the familial susceptibility for cancer are less well understood than those for inherited or genetic cancers. Cancers that tend to run in families include breast, colorectal, and prostate cancers. The impact of the environment in determining whether an individual with a familial susceptibility for cancer will actually develop cancer is not completely understood. Most of the researchers believe

that lifestyle and environmental factors markedly influence whether a person with a familial susceptibility for cancer will develop cancer. Therefore, even with an increased familial susceptibility for certain cancers, a person can modify his or her risk for developing this form of cancer by changing the environment or lifestyle practices.

Gender factors

Certain cancers are selective for a given gender due to the presence of a gender-specific organ, e.g. cancer of the uterine cervix in females and cancer of the prostate in males. However, for other cancers with a common target, there exists a definite dichotomy in epidemiological features:

the majority of tumours have a higher incidence and mortality in males than females;

only tumors of breast, gallbladder, right side of the colon, genital tract and thyroid have a higher incidence in females;

breast cancer is many hundreds of times more common in females than males, presumably because of the average greater volume of tissue which has a sustained exposure to physiological hormonal cycles;

lung cancer in females is also increasing, probably because of an increase in smoking in this group; however, it is still less than male rates and this may be partly due to a genetic predisposition: lung cancer rates in male non-smokers are approximately 50% greater than female non-smoker counterparts.

Age factors

Although environmental factors play an important role for cancer development, age is considered as one of the major predictors increasing the cancer risk - the general rule is the following: with increasing age there is an increase in the incidence of cancer. Rates for the development of cancer begin to increase at 40 years of age and then increase rapidly at 50 years of age. This has been attributed to a longer duration for genes to mutate by chance or to be acted upon by carcinogens - a cumulative effect.

Examples of cancers which follow this trend are those of the gastrointestinal and urinary tracts. However, there are some notable exceptions:

- the second most common cause of mortality in the infant and childhood group is cancer, e.g. neuroblastoma and Wilms tumour, and for these there is usually a strong inherited component
- seminomas and teratomas peak in incidence before 30 years of age and then fall to minimal incidence
- osteosarcoma has a bimodal distribution; it is common in adolescence when bone growth is rapid and also after 45 years of age as a consequence of Paget's disease
- uterine cervical carcinoma arises with increasing frequency in adolescence and then increases relatively slowly in incidence until the menopause when it again declines

Geographical factors

The location of a population may have a profound influence on its incidence of a given cancer. Standardized tumor registries have allowed detailed comparisons. Some commonly quoted examples include: the mortality from stomach cancer is 7 times higher in Japan than the United States, the incidence rate of skin cancer is 200 times higher in northern Australia than India, the incidence rate of lung cancer is 35 times higher in England than Nigeria

For these examples, it is thought that exposure to an environmental agent in a particular location results in the increased risk, e.g. sun exposure in Australia. In support of this is the evidence from migrant studies. Immigrants commonly acquire with successive generations the risk for a given cancer related to their new location.

Yet, it is often hard to extricate geographical factors from other differences, which characterise communities, e.g. ethnic origin or national conventions. Hence, Mormons in Utah in the U.S. have a lower incidence of respiratory, gastrointestinal and genital cancer than other communities in the same state. Equally, the death rate from breast cancer is higher in Denmark than Sweden despite their close apposition; social differences in the number or pregnancies and breast feeding may have a role.

Social factors

Social factors are intimately tied to issues such as race, economic status and religion. Environmental exposures and lifestyle practices have been determined to be the risk factors in the development of cancer. The major lifestyle factors that contribute to cancer include smoking, alcohol, diet, medical practices, and ultraviolet exposures. Generally, it is difficult to consider that behaviour of one group predisposes them to a particular set of cancer, but there are some exceptions:

- tobacco consumption - smoking, chewing: polycyclic hydrocarbons in tobacco are potent carcinogens and have been linked to a number of cancers including those of the buccal cavity, nasopharynx, esophagus, bladder and cervix. More than 30% of all cancer deaths are directly related to smoking.
- alcohol consumption has been linked to increased rates of cancer in the upper respiratory tract, digestive tract, breast, colorectum, and liver. The mechanisms

for increased rates of breast cancer from alcohol consumption are unclear but may be related to impairments in the immune function or the inability of the liver to clear the body of carcinogens, or from decreases in cell membrane permeability in the breast. For colorectal cancers, alcohol consumption has been shown to increase

- rectal cell proliferation or growth in the rat. This increase in the proliferation of rectal cells from alcohol exposure may be the mechanism involved in the promotion of colorectal cancers. Alcohol combined with tobacco usage has also been shown to contribute to increased rates of cancer in the mouth, pharynx, larynx, esophagus, and liver.
- dietary make-up: Dietary practices and obesity have been linked to certain types of cancer.

Colon carcinoma is more common in the developed world; it is suggested that the low-residue diet in this setting prolongs the time in which faecal carcinogens are in contact with the bowel wall.

Over-salting of fish in South China may contribute to nasopharyngeal carcinoma. There are also high rates of stomach cancers in countries where large quantities of salted fish or similarly preserved foods are consumed.

Methods of food preparation may also increase cancer rates. Excessively smoked or broiled fish or meat, or repeatedly reused fats for frying foods release Benzo(a)pyrene and other polycyclic hydrocarbons that may potentially cause cancer.

High consumption of dietary fat may increase bile acids and cholesterol metabolites that may increase carcinogens in the body that are associated with colorectal cancers.

Low fiber diet have also been linked to increased rates of colon cancer.

Food additives and food preparation are also suspect as cancer-causing agents. Nitrates, salts, and saccharin have been investigated as possible carcinogenic substances. Saccharin has not been shown to increase cancer risk in humans; however, this is not the case for nitrates and salts. Nitrates and salts that are used to preserve foods appear to increase rates of glandular stomach cancers.

Therefore, dietary guidelines associated with lowering the risk of cancer include increasing the use of fiber, fruit, and vegetables in the diet, limiting alcohol consumption, and limiting foods that contain preservatives, or foods that are grilled or blackened (American Cancer Society, 2000).

Obesity is associated with an increased incidence of colorectal carcinoma, possibly due to the secretion of excess amounts of bile acid. Obesity caused by a sedentary lifestyle and/or a high consumption of dietary fat appears to contribute to an increased risk for cancers of the breast, the ovaries, and the endometrium in females (National Heart, Lung, and Blood Institute, 2000). Obese females have higher numbers of fat cells, and fat cells produce estrogen. Since higher levels of estrogen have been associated with higher levels of endometrial, ovarian, and breast cancers, it has been suggested that higher levels of estrogen from increased numbers of fat cells in obese females may increase their cancer risk.

Medical practices and drugs have also been linked to increases in cancer rates. Androgen -anabolic steroids used to promote athletic performance and prevent muscle wasting cause cancers in the liver, prostate, and breast. Estrogen replacement

medications and steroid contraceptives may contribute to increased risk for developing cancers of the endometrium, vagina, ovaries, and breast. Immunosuppressants, such as those used in transplant procedures, are linked to lymphomas, skin cancer, and soft tissue sarcomas. Interestingly, some chemotherapeutic drugs used to treat cancer, such as alkylating agents, are linked to cancers of the bladder and to leukemia. In situations where long-term prognosis is a factor, the benefits must be weighed against the risks when choosing to use these drugs.

MECHANISMS OF ANTI-TUMOR RESISTANCE

There are following mechanisms (steps) to prevent the appearance of the tumor cells and further tumor development:

1) Anti-carcinogenic mechanisms prevent the carcinogens entry into the cells and their further action on the cell genome.

Chemical carcinogens can be inactivated through:

a) physical and chemical fixation (oxidation, reduction, methylation etc.);

b) engulfment of carcinogens (phagocytosis) with their following inactivation.

Physical carcinogens can be inactivated via:

a) inactivation of oxygen radicals with antioxidant enzymes (superoxidedesmutase, catalase) or non-enzyme pathway (tocopherols – vitamin E; glutatione system);

b) enzyme-mediated digestion (catalase, glutationperoxidase, glutationreductase etc.).

Biological carcinogens can be inactivated by immunoglobulins preventing the virus entry into the host cells.

If the anti-carcinogenic mechanisms became unsufficient, carcinogen might induce the mutations in the genome, which might affect the proto-oncogens (after being mutated and activated they are considered as a c-onc).

2) Anti-mutational mechanisms include detection, elimination and suppression of oncogens activities. This can be due to: a) activation of tumor-suppressor genes; b) activation of DNA repair pathways.

If the anti-mutational mechanisms became unsufficient, the cells harboring the carcinogen-induced mutations became transformed into the tumor cells which have the specific tumor-related phenotype (ability to uncontrolled growth, invade the surrounding tissues, to make the metastasis, etc.)

3) Anti-cellular mechanisms include the detection and killing of the tumor cells.

This can be due to: a) the immune mechanisms - cytotoxic T-lymphocytes and natural killers; b) non-immune mechanisms (TNF-α: oncolytic activity is mediated by

the ability to induce thrombosis in tumor vessels thereby inducing the ischemia and following tumor necrosis and induce the secretion of cytokines with potent anti-tumor activities (IFN-α, β; IL-6); α-LP – has the potent oncolytic activity.

Cancer therapy

Surgery (local); chemotherapy (systemic); radiotherapy (local); endocrine therapy (systemic); immunotherapy (local-systemic). The detailed mechanisms of action of the radiotherapy, endocrine therapy and immunotherapy and each class of chemotherapeutic drugs as well will be discussed on the seminars.

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