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Dedication

To the one who gave me everything, her love, care, and patience, the owner of the purest heart, my dear ***mother****. Thank you, Mom, for raising me well and for being the guiding light in my life.*

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Index

Figures list:

Abbreviations list:

- 3FTx: Three-finger toxin
- ADCC: Antibody-Dependent Cell-Mediated Cytotoxicity
- APC: Antigen Presenting Cell
- Bbill-TX: Bothrops billilineata Toxin
- Bl-PLA2: Bothrops leucurus Phospholipase A2
- CA: Crotapotin Subunit A
- CB: Crotapotin Subunit B
- CRP: C-reactive protein (acute-phase response protein)
- CTX: Cardiotoxin
- CVF: Cobra Venom Factor
- ECM: Extracellular Matrix
- ERK1/2: Extracellular Signal-Regulated Kinase 1/2
- Fc: Fragment crystallizable (region)
- G2: Fraction of Flavodoxin
- GM-CSF: Granulocyte-Macrophage Colony-Stimulating Factor
- H2O2: Hydrogen Peroxide

- IFN-γ: Interferon Gamma
- IgA: Immunoglobulin A
- IgD: Immunoglobulin D
- IgE: Immunoglobulin E
- IgM: Immunoglobulin M
- IKK : IκB Kinase
- IL-1 : Interleukin-1
- IL-10 : Interleukin-10
- IL-12 : Interleukin-12
- IL-17 : Interleukin-17
- IL-18 : Interleukin-18
- IL-1α : Interleukin-1 Alpha
- IL-1β : Interleukin-1 Beta
- IL-4 : Interleukin-4
- IL-6 : Interleukin-6
- IL-8 : Interleukin-8
- iNOS : Inducible Nitric Oxide Synthase
- LAAOs: L-amino acid oxidases

- LFA-1: Lymphocyte Function-Associated Antigen 1
- LTB4: Leukotriene B4
- LXA4: Lipoxin A4
- MAPK: Mitogen-Activated Protein Kinase
- MHC: Major Histocompatibility Complex
- MMP : Matrix Metalloproteinase
- MOMP : Mitochondrial Outer Membrane Permeabilization
- MPO: Myeloperoxidase
- MyD88: Myeloid Differentiation Primary Response 88
- nAChR: Nicotinic Acetylcholine Receptor
- NF-κB: Nuclear Factor Kappa B
- NO: Nitric Oxide
- PGE2: Prostaglandin E2
- PLA2: Phospholipase A2
- ROS: Reactive Oxygen Species
- SIRS: Systemic Inflammatory Response Syndrome
- sPLA2: Secreted Phospholipase A2
- SVMP: Snake Venom Metalloproteinase

- TGF-β: Transforming Growth Factor Beta
- Th1: T Helper 1 Cells
- Th2: T Helper 2 Cells
- TLE: Thrombin-like enzyme
- TLR: Toll-Like Receptor
- TNF: Tumor Necrosis Factor
- TNF-α: Tumor Necrosis Factor-alpha
- TRAIL: TNF-related apoptosis-inducing ligand
- Ts1: Toxin from Viperidae Snake Venom
- TWEAK: TNF-like weak inducer of apoptosis
- VLA-4: Very Late Antigen-4

Introduction

Introduction

Snake venom is a fascinating and complex substance, often shrouded in mystery and intrigue. Produced by specialized glands in the heads of certain snake species, venom serves a crucial role in subduing prey, aiding in digestion, and defending against predators. It's a potent cocktail of proteins, enzymes, and other bioactive molecules meticulously crafted by evolution to fulfil various purposes in the snake's life.

While the composition of snake venom varies widely between species, it typically contains a mixture of toxins tailored to target specific physiological systems in their prey. Some venoms primarily attack the nervous system, causing paralysis and immobilization, while others target the blood, tissues, or organs, inducing symptoms ranging from haemorrhage to organ failure.

Understanding snake venom is not only crucial for appreciating the marvels of natural adaptation but also for its practical applications in medicine and scientific research. Through studying venom, scientists have developed life-saving antivenoms, unraveled the mysteries of biochemical pathways, and even discovered novel drugs with therapeutic potential.

Chapter one

Zoological and anatomical description of snake

Introduction

Snakes, with their sinuous bodies, forked tongues, and often mysterious demeanor, captivate the imagination of people worldwide. As a diverse group of reptiles, they inhabit a wide range of ecosystems, from lush rainforests to arid deserts, and play essential roles in maintaining ecological balance. With over 3,000 species found on every continent except Antarctica, snakes exhibit remarkable diversity in size, coloration, behavior, and habitat preferences. From the massive anaconda, capable of swallowing prey whole, to the tiny thread snake, scarcely longer than a pencil, snakes come in an array of shapes and sizes. Snakes are well-adapted predators, equipped with specialized features such as heat-sensing pits, infrared vision, and hinged jaws that allow them to consume prey much larger than their heads. Many species rely on venom to immobilize or kill their prey, while others constrict their victims with powerful coils. Despite their often-misunderstood reputation, snakes play vital ecological roles as both predators and prey. They help control populations of rodents and other pests, regulate ecosystems, and serve as a food source for many other animals. throughout history, snakes have been symbols of both fear and reverence in various cultures. From ancient myths and legends to modern-day symbolism, snakes evoke a wide range of emotions and interpretations [21].

1. Snake Anatomy

Due to their essentially elongated tubular structure, snakes' main anatomical parts can be conveniently categorized into distinct sections:

- When laid out straight on a table with the head on the left, progressing from left to right, the initial 25 percent of the snake encompasses the head, esophagus, trachea, and the heart, constituting the major organs and components.
- Moving into the second quarter, approximately 26 to 50 percent of the snake includes the upper lungs, liver, and, positioned three-fourths down from the head, the stomach.

- In the third quarter, spanning about 51 to 75 percent of the snake's length, one encounters the gall bladder, spleen, pancreas (or splenopancreas, depending on the species), and the gonads (testes or ovaries). The small intestine courses between these structures, accompanied by the right lung (and in some species, the left lung as well).
- In the final quarter, ranging from 76 to 100 percent of the snake's length, one finds the junction between the small and large intestine, the cecum (if present), the kidneys (right in front of the left), and the cloaca. Familiarizing oneself with this anatomical roadmap for snakes contributes to a more adept understanding of herpetology [9].

2. Habitat

Our native snake species are found in a diverse range of environments, including fields, forests, wetlands, ponds, lakes, streams, rocky hillsides, farmland, vacant lots, and residential neighborhoods. Within these varied habitats, snakes display versatile behaviors such as traveling along the ground, swimming, climbing trees and bushes, and occasionally exploring subterranean areas. It's worth noting that while some snakes may engage in burrowing, the majority of what are commonly referred to as "snake holes" are actually created by small mammals like chipmunks, mice, and shrews. Although certain snake species utilize these burrows for purposes such as finding food, seeking shelter, and laying eggs, it's important to emphasize that most snake species do not engage in hole-digging activities [12].

3. Food Chain

Snakes play pivotal roles in natural ecosystems, contributing to the "balance of nature" as both predators and prey. All snake species operate as predators, with their diet varying based on size and species. They may feed on invertebrates like slugs, worms, and insects, or larger prey such as fish, amphibians, snakes, birds, bird eggs, and small mammals.

Certain species, like the milk snake and black rat snake, provide substantial benefits to farmers by consuming significant numbers of rodents. The milk snake, in particular, demonstrates its utility by entering burrows and preying on young mice and rats within nests. Garter, redbelly, and brown snakes often target garden pests like slugs and soft-bodied insects. Snakes employ sight, scent, and temperature to locate their prey. Their exceptional sense of smell is facilitated by a constantly flicking forked tongue that carries scent particles to a specialized sensory organ known as "Jacobson's organ" on the roof of the mouth. Hunting methods vary among species, encompassing pursuits, ambushes, and scavenging. Some species use venomous bites, while others rely on constriction or sheer overpowering before swallowing prey whole. Despite lacking chewing teeth, snakes ingest their meals whole. The feeding frequency of native snake's ranges from several times a day to once a month, depending on meal size and habitat temperature. In the intricate web of the food chain, snakes and their eggs become targets for various predators, including fish, amphibians, other snakes, birds, and mammals like skunks, raccoons, and opossums. Notably, birds, encompassing not just hawks and owls but also songbirds, emerge as significant predators, often consuming a considerable number of small snakes. It's not uncommon to witness the tail of a young garter snake protruding from the well-fed gullet of a nestling robin [12].

4. Locomotion and Swimming

4.1. Locomotion

Snakes exhibit four distinct modes of movement, each perceived as equally graceful and intriguing due to their limbless structure. Despite the appearance of rapid motion, snakes do not exceed speeds of 5-6 km per hour, and this pace is not sustainable over prolonged periods. This perceived swiftness may be a result of panic-induced misjudgment on our part.

- **Serpentine or Lateral Progression:** Commonly known as "slithering," this undulating crawl is the most prevalent and efficient form of movement. All water snakes utilize this technique for swimming, allowing them to achieve maximum speeds.
- **Rectilinear or Caterpillar Movement:** Larger, heavier snakes employ a caterpillar-like motion to travel in a straight line. They use powerful muscles to move the scutes forward and then pull the rest of the body along. This method is also advantageous when climbing trees.
- **Sidewinding**: In sidewinding, the snake's body makes contact with the ground only at specific points, creating an S-shaped curve as it transitions between contact points. Snakes in desert environments with loose sand employ sidewinding to maneuver easily across flat, low-friction terrain. A variation called saltation involves the body straightening so forcefully and rapidly that it lifts entirely off the ground, serving as a rapid escape movement.
- **Concertina:** Snakes use the concertina technique when climbing trees, forming horizontal loops as the body bunches up, and then extending forward as the head moves, resembling the motion of an accordion or spring [21].

4.2. Swimming

Snakes have effectively capitalized on aquatic environments due to the abundant food resources attracted to water, making it an advantageous habitat. Every snake possesses the ability to swim, with aquatic snakes exhibiting exceptional proficiency in the water. Their movements in aquatic settings are characterized by grace and swiftness. Notably, their eyes and nostrils are positioned higher on the head, enabling them to breathe and see without the need to raise their heads above the water surface. Sea snakes, in particular, feature flattened tails that function as paddles, propelling them through the water with ease [21].

5. Classification of snake

From a scientific perspective, a snake is essentially a constituent of the animal kingdom, possessing a backbone and exhibiting the typical traits of a reptile. All snakes are identified like this:

Kingdom	Animalia
Phylum	Chordata
Sub-phylum	Vertebrata
Class	Reptilia
Order	Squamata
Suborder	Serpentes /Ophidia

There are over 2600 species of known snakes, just 450 of them are venomous and only about 270 species have a fatal venom to human being. the four dangerous family because of their venoms are:

- **Colubridae:** Known as the Aglypha (fig01), because their teeth are quite solid, they have grooved fangs at the posterior end of the upper jaw. Showing no traces of grooving such as boomslang, tree snake, vine snake etc (not all colubridae are venomous) their venom effect the blood.

Figure 01: snake from colubridae family [21]

- **Hydrophidae:** It includes all sea snake belong to the division of snakes known as the Proteroglypha, because they have fangs in the front part of the upper jaw such as Black and Yellow Sea Snake their venom effect is on the nervous system and cause paralysis (fig02).

Figure 02: snake from Hydrophidae family [21]

- **Viperidae:** The viper family of snakes are different in appearance to those of the Colubrine family, to which the Cobra belongs (fig03). They have fiat heads, usually more or less triangular, bodies thick and plump, and tails short such as Vipers, Adders, bushmasters and cop Banded Copperheads their venom effect the blood, heart, and cause severe damage to the skin and muscles near the bite.

Figure 03: snake from Viperidae family [12]

- **Elapidae:** Having fangs set in the front part of the upper jaw, in the bones known as the anterior maxillary bones (fig4). The fangs are usually deeply grooved or channelled. In some Species he sides of the grooves Show a tendency to unite and form, it includes cobra, kraits, mambas their venom cause swelling, blistering, and damage to the skin near the bites [21].

Figure 04: snake from Elapidae family [9]

6. The systems of snake

6.1. Respiratory System

Snakes feature a small opening situated just behind the tongue known as the glottis, which connects to the trachea or windpipe. In contrast to mammals, the reptile glottis remains consistently closed, forming a vertical slit except when the snake breathes. A piece of cartilage within the glottis vibrates when the snake forcefully expels air, producing the characteristic hiss. Snakes can extend their glottis out the side of their mouth during feeding, allowing respiration while consuming large prey.

The trachea resembles a long, straw-like structure supported by cartilaginous rings, forming an incomplete "C" shape, akin to lizards. The trachea typically terminates just in front of the heart, splitting into two primary bronchi directing air into the left or right

lung. Most snakes have a vestigial left lung connected to the short-left bronchus (fig05), while the right bronchus terminates in the functional right lung. Snakes primarily breathe by contracting muscles between their ribs, lacking a diaphragm as seen in mammals.

The respiratory exchange occurs in the portion of the snake's lung nearest its head, while the portion near the tail functions more as an air sac, devoid of respiratory gas exchange [9].

6.2. Cardiovascular System

Snakes possess a three-chambered heart comprising two atria receiving blood from the lungs and body, and a large ventricle pumping blood into arteries. Despite being evolutionarily less advanced than the mammalian four-chambered heart, the snake's heart functions similarly due to divisions and valves within the ventricle [9].

An intriguing adaptation in the snake's cardiovascular system is the renal portal system, where blood from the tail passes through the kidneys before returning to the general circulation. This system can impact the effectiveness of drugs, especially when administered through the tail or rear legs [9].

6.3. Immune and Endocrine Systems

Unlike mammals, snakes lack lymph nodes, making illness less visually apparent. Some species have tonsil-like tissue in the esophagus. The spleen, responsible for red blood cell functions, is closely associated with the pancreas, collectively referred to as the "splenopancreas."

The pancreas, a major endocrine organ, regulates blood-glucose levels and produces digestive enzymes. Snakes lack a gall bladder association with the liver, unlike mammals. The thymus, found in front of the thyroid gland, produces immune cells. The thyroid gland regulates metabolism and the shedding cycle, while reptiles have one or two pairs of parathyroid glands regulating calcium and phosphorus levels.

Adrenal glands, often called "stress glands," are located near the gonads and urogenital structures, producing corticosterone during stress, suppressing the immune system and increasing susceptibility to disease in snakes [21].

6.4. Digestive System

The digestive process in snakes is remarkably efficient, as it dissolves and absorbs all components of the prey except for hair and claws. The bones of the prey undergo complete digestion within 72 hours, yet snakes require additional time for thorough assimilation of their food.

The digestive systems of snakes that intermittently but heavily feed differ from those of more regularly feeding counterparts. In intermittent feeders, there is a notable increase in metabolic rate, nutrient transport across the gut wall, and even the mass of intestinal tissue compared to the resting stage. After digestion, the gut transitions into an inactive stage, particularly in snakes that feed less frequently. This ability to regulate the digestive system based on food availability is believed to have evolved as an energy-conserving mechanism, especially in vipers and pythons with infrequent feeding patterns.

Following a meal, snakes enter a torpid state as the digestion process unfolds. The noticeable bulge in the mid-section of a snake after a meal is apparent even to casual observers. Instances of bloated snakes stranded on roads necessitate human intervention to relocate them to safer areas for digestion away from potential dangers.

The snake's digestive system comprises the esophagus, stomach, small intestine, colon, and associated digestive glands. Unlike in some animals, the snake's esophagus lacks musculature, necessitating the assistance of the entire body's movements to propel food to the stomach. The junction between the esophagus and stomach is not well-defined. The stomach features interior longitudinal folds to enhance the surface area for digestion and absorption. The liver, gall bladder, and pancreas are present, with the liver being the largest internal organ, secreting bile. The gall bladder stores and releases bile

into the small intestine when needed. The pancreas secretes digestive enzymes and hormones regulating blood sugar.

The small intestine acts as a lengthy tube receiving and absorbing nutrients from the stomach, transporting them to the large intestine. Fecal matter is then conveyed to the cloaca, a common chamber receiving products from the digestive, urinary, and reproductive systems, ultimately expelled through the cloacal opening [21].

6.5. The sensory system:

- **Vision**: Snakes possess one eye on each side of their head, extending their field of vision. Their eyes lack lids and remain perpetually open. Most snakes exhibit keen eyesight, with the ability to detect motion. Arboreal or tree-dwelling snakes typically have superior vision, while burrowing species fare less well in this regard. In the majority of snakes, the lens moves back and forth within the eyeball for focusing. Diurnal snakes feature rounded pupils, whereas nocturnal ones have vertical pupils akin to cats. Beyond their eyes, some snakes have infrared-sensitive receptors in deep grooves between the nostril and eye, enabling them to perceive radiated heat. Snakes equipped with pit organs can detect temperature differences as subtle as 0.5 degrees Celsius. Pit vipers employ these pits to locate prey by sensing the body heat emitted. Leveraging this exceptional heat-seeking ability, pit vipers can even pursue and capture prey animals in complete darkness [21].
- **Sense of hearing:** The expression "Deaf as an adder" finds its validity in the fact that adders, being snakes, lack external ears, rendering them entirely devoid of the ability to hear airborne sounds. This deafness arises from their incapacity to pick up such sounds. However, certain modifications in the bones of their head endow adders with an extraordinary sensitivity to ground vibrations. The body parts in direct contact with the ground can perceive these vibrations, enabling snakes to sense the approach of other animals [21].

- **Sense of smell:** Accusing someone of duplicity is often expressed with the phrase "Speaking with a forked tongue." This sentiment reflects a prevalent bias against snakes, but it is intriguingly rooted in reality, as snakes do possess forked tongues. Additionally, they feature a notch on the upper lip, enabling the tongue to flick in and out while the mouth remains closed. The rhythmic movement of their forked tongues serves a crucial purpose, as snakes employ them for smelling. During this olfactory process, a snake collects airborne particles on its forked tongue and then transfers them to either the Jacobson's organ or the Vomeronasal organ in the roof of the mouth for meticulous examination. These organs analyze the chemical composition of the molecules in the air, and the resulting information is relayed to the snake's brain. The forked structure of the tongue allows the snake to "differentiate" or compare sensations received by the two ends, providing the snake with a type of "stereo or directional sense" of smell. This sensory ability enables the snake to discern the richer sensation, automatically determining the direction to follow prey, even in complete darkness, and precisely timing when to strike. The fundamental principle is comparable to humans having two ears, allowing us to identify the source of sounds. Remarkably, the snake's tongue serves a dual purpose as a touch-sensing organ, delicately probing the surroundings. Intriguingly, snake tongues seem to lack taste buds in their sensory repertoire [21].

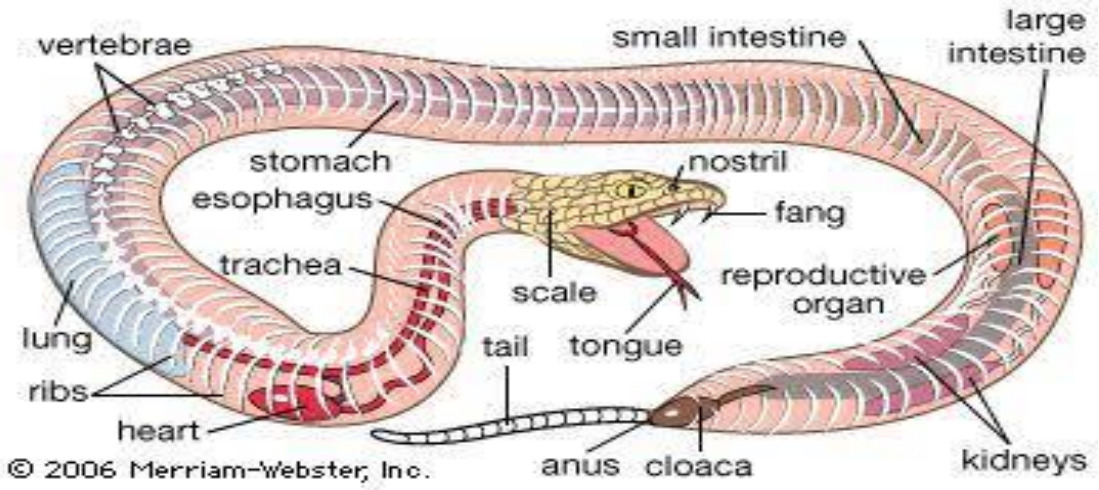

Figure o5: Anatomical systems of snake [21]

Chapter two

The venom

Introduction

Snake venoms are complex mixtures which play adaptive roles associated with prey immobilization and digestion, as well as with defense against predators. Snake venom evolution has involved the recruitment of diverse genes and their duplication and expression in venom glands. Proteomic analysis of snake venoms has demonstrated the high complexity of these secretions. Toxins in snake venoms correspond to proteins of both enzymatic and non-enzymatic nature, which exert many different deleterious effects in the organism [2].

1. The Venom Apparatus

The snake venom apparatus is a complex device consisting of a specialized gland that synthesizes a toxic secretion, situated in the temporal region behind the eye, a duct with an accessory gland, muscles for squeezing the venom, and fangs for delivering the toxic venom (fig 06). Which can inject the venom into the body of the prey. The venom gland is a modified salivary gland, and is located just behind and below the eye. The size of the venom gland depends on the size of the snake. This structure is particularly complex in snakes. The venoms have derived from a specialization of digestive secretions, pancreatic, certainly salivary, which originally led to the digestion of the tissues. The saliva has a twofold role: it lubricates the food and it starts the process of digestion. Later the venoms would have developed the capability to kill and to immobilize the prey with the help of specialized toxins facilitating holding and swallowing, which are difficult without the help of legs [2].

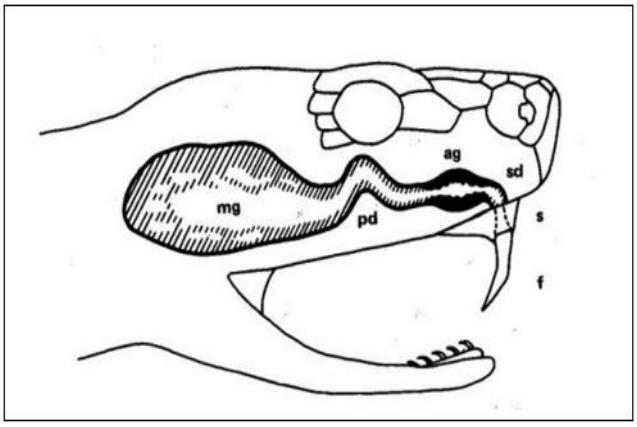

Figure 06: schematic drawing of the venom apparatus. venom is synthesized and stored in the main venom gland (mg) and transported by the primary duct (pd) through the accessory gland (ag) and secondary duct (sd) which exits into the fang (f). the loop in the primary duct accommodates the movement of the fang. All of these features occur bilaterally [2]

2. Venom

2.1 Properties of snake venom

Snake venom is a combination of protein substances and salts dissolved in from 65 to 80 percent of water. It is a slightly acidic fluid with a consistency similar to glycerine, displaying varying degrees of yellow tint based on the snake species. on exposure to the air and light, it gradually loses its fluidity. but does not crystallize, being of a volatile nature so he will disappear, leaving the dry venom neutral. but, if venom be mixed with glycerine, it will keep indefinitely It is obtained by manual pressure on or electrical stimulation of the salivary glands of the snakes. Snake venoms exhibit a high degree of complexity and diversity in their effects. The composition, nature, and impact of venoms vary significantly. Notably, the acidity in the venom induces a stinging sensation in the wound caused by the fangs and aids in the swift absorption of the venom [2].

2.2 The biochemical composition of the snake venom

Conventionally, proteins extracted from snake venoms are categorized into two groups: enzymes, which typically have low acute toxicity, and toxins, whose pharmacological roles are better known and the ratio of these component et are different from snake to another depends on their family (Fig 7) [2].

2.2.1 Enzymes

The enzymes are proteins with elevated molecular weights. The catalytic properties that set them apart from toxins result in two significant outcomes. the degradation product crucial for toxicity lacks immunogenic properties in the recipient organism, thereby preventing the synthesis of specific antibodies. Secondly, the toxicological effects are more contingent on the duration of the enzymatic reaction than on the initial quantity of enzymes. They induce a biological transformation without undergoing any modifications themselves, enabling them to continue reacting as long as they remain present in the organism. The specificity of enzymes in snake venoms varies [2].

- **Phospholipases:** In snake venoms the Phospholipases A2 are the most common. it causes hydrolyse free phospholipids and those bound to membranes, into fatty acids and lysophospholipids. also affects several physiological systems depending on the type of hydrolysed phospholipids, haemostasis, neuromuscular transmission and inflammatory reaction.

- **Acetylcholinesterase**: The Elapidae feature an acetylcholinesterase capable of hydrolysing acetylcholine. This enzyme plays a role at the synapse by facilitating the transmission of nervous stimuli to the postsynaptic membrane. It contributes to the intricate neurotoxic effects observe in elapid venom.

- **Phosphoesterases:** Many venoms contain various phosphoesterases. The endonucleases cleave nucleic acids (both DNA and RNA) at the bonds connecting the base pairs. The exonucleases target the terminal base of the nucleic chain. The phosphodiesterases sever the connection by eliminating the oxygen from position 3' of the ribose or deoxyribose, thereby separating them from the phosphorus. The phosphomonesterases exhibit lower specificity, breaking down all mononucleotides, specially those implicated in cellular energy transport [22].

- **L-amino-acid-oxidase:** L-amino acid oxidases (LAAOs) exhibit multifunctional properties, producing hydrogen peroxide and ammonia as integral components of their catalytic processes. These by-products possess high toxicity, capable of disrupting crucial cellular components such as nucleic acids, proteins, and the plasma membrane. Extracellularly, these enzymes initiate the production of highly toxic oxygen reactive species. The resultant hydrogen peroxide and ammonia, being potent agents, modify the permeability of the plasma membrane, triggering apoptosis and subsequently culminating in cell death. The yellow colour of the venom is attributed to the flavin-adenine-dinucleotide group attached to this enzyme [23].

- **Proteases**: Numerous enzymes affect the structure of proteins, and Viperidae venoms are particularly rich in them. These proteases can be classified into:

- Serine proteases: are glycoproteins, either single- or double-chained, that bind to their substrate using a serine. includes the majority of thrombin-like enzymes, as specific fibrinolytic enzymes and fibrinogenolytic enzymes responsible for degrading the B13 chain of fibrinogen. Once activated, it breaks down the fibrin mesh into soluble fibrin degradation products which are removed from the plasma.

- Metalloproteases: existing as single or double chained proteins, require the presence of a metal ion, typically zinc, for their functionality. Calcium is also essential for ensuring their structural stability. These enzymes are highly responsive to pH changes and become inactive in an acidic environment They activate the factor X, and their catalytic activity targets the vascular endothelium [2].

- **Hyaluronidase:** It hydrolyses hyaluronic acid or chondroitin sulphate, which are mucopolysaccharides crucial for the cohesion of connective tissue. As a result, this enzyme promotes the dispersion of venom following its injection through a bite [22].

2.2.2 Toxins

- Toxins are proteins with varying molecular weights, typically below 30 kDa, making them smaller than enzymes. They possess the capacity to selectively bind to specific receptors, commonly located on a membrane. The tropism of toxins may manifest as neurological, cardiovascular, muscular, or undifferentiated, contingent upon the anatomical distribution of the recognized receptors The toxicological impact is determined by the relationship between the administered toxin quantity and the amount of corresponding receptors, defining it as dose-dependent. The venoms of the Elapidae are particularly rich in toxins, categorized in three groups [14]:

- **Cardiotoxin**

CTXs disrupt the membranes of neurons, skeletal muscle cells, and cardiac muscle cells. They induce the formation of pores in cell membranes, resulting in depolarization and calcium influx, leading to muscle contraction, cell lysis, and cardiac arrest. Ventricular tissues exhibit high sensitivity to CTXs, causing myonecrosis in skeletal

muscle cells, which leads to rapid myoblast degeneration, myofibrillar clumping, and hypercontractility of sarcomeres. Additionally, CTXs contribute to necrosis in muscle fibres and damage to nerve cells. In a study by Shear et al., gene expression analysis of mice exposed to a lethal dose of CTX from N. sputatrix revealed the most significant changes in gene expression occurred in the heart. These changes affected genes involved in inflammation, apoptosis, energy metabolism, and ion transport [14].

- **Neurotoxin**

- **three-finger toxin (3FTx) super family:** The majority of toxins found in snake venom are part of the three-finger toxin family, named for the distinctive protein fold characterized by three β strands. While the superfamily of three-fold proteins is present in all eukaryotes, these 3FTxs are specific to snakes. They exhibit a unique binding affinity for post-synaptic sites, leading to the induction of flaccid paralysis in the prey. Notably, unlike other snake toxins associated with inflammation and hyperalgesia, 3FTxs do not contribute to these effects. The versatility of the 3-finger fold allows these toxins to target a variety of ion channels and receptors in the prey, showcasing their ability to modulate diverse biological functions.

- **Nicotinic Acetylcholine Receptor Toxins:** Bind to and inhibit nAChRs on postsynaptic membranes are present as a-neurotoxins in Elapidae and Hydrophidae snakes, these toxins give rise to a block of neuromuscular transmission eventually leading to death by asphyxiation. These toxins are specific, competitive inhibitors of acetylcholine at nAChRs, multi subunit ligand-gated ion channels on the postsynaptic membrane. The toxins decrease the ability of acetylcholine to depolarize the postsynaptic cell. a-Neurotoxins bind to the highly conserved a-subunit of the nAChR, preventing opening of the nAChR-gated Na channel and giving rise to blockage of electrical transmission [14].

- **Hemotoxin**

- **Disintegrins:** A group of compact, non-enzymatic polypeptides ranging from 40 to 100 amino acids, are potent antagonists of integrin receptors. Their ability to compete with fibrinogen for the aIIbb3 integrin receptor allows disintegrins to effectively hinder platelet aggregation. Consequently, disintegrins play a crucial role in inhibiting the formation of the platelet plug and impeding the necessary platelet aggregation for hemostasis. This interference leads to sustained bleeding, which can exacerbate the detrimental effects of the venom.

- **Procoagulants:** The conclusive common route of the coagulation cascade relies on Factor Xa, an endopeptidase that transforms prothrombin into thrombin. Thrombin, a crucial protease, assumes three major functions. It converts fibrinogen into fibrin monomers, triggers Factor XIII activation responsible for interlinking fibrin polymer chains into an insoluble mesh-like complex forming the foundational structure of a blood clot. Additionally, it activates Factors V and XI, amplifying thrombin activation through a positive-feedback loop. Procoagulant toxins induce blood clotting through diverse mechanisms, classified into categories such as TLEs, prothrombin activators, Factor X activators, Factor V activators, and other activators, depending on their specific targets within the cascade [2].

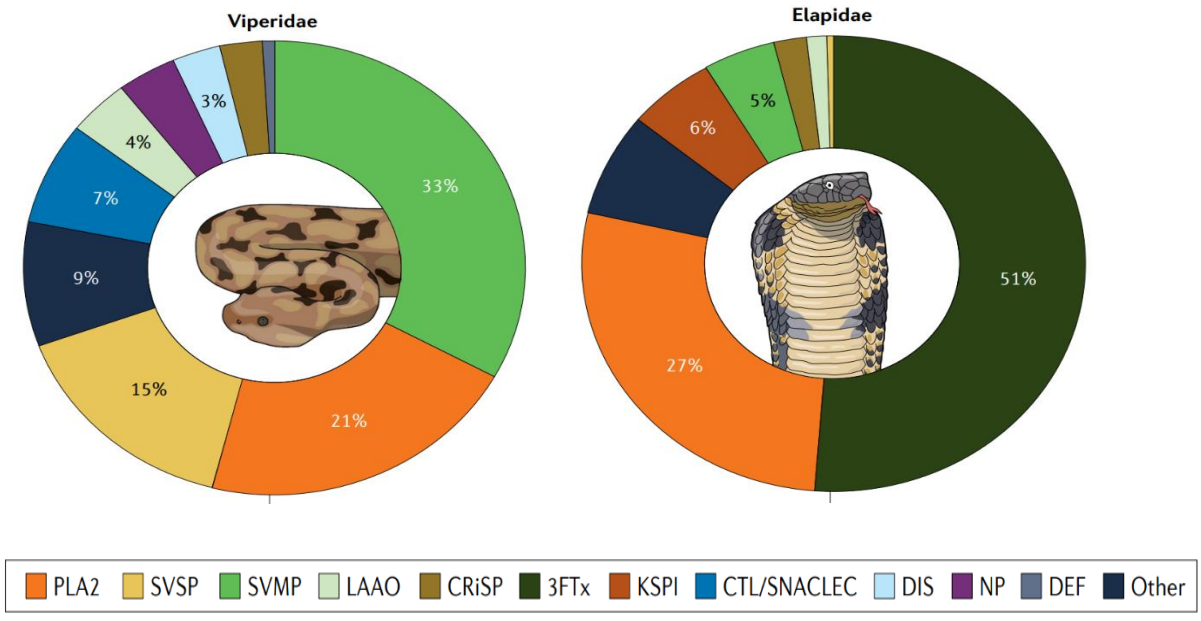

Figure 07: Relative circles represent the ratio of toxins in the most dangerous family of snakes [20]

Chapter three

Immune cells involved

Introduction

The immune system is a complex network of cells, tissues, and organs that work together to defend the body against harmful pathogens such as bacteria, viruses, fungi, and parasites. Its primary function is to identify and eliminate these invaders, as well as to detect and destroy abnormal cells, including cancerous ones. The immune system is essential for survival, providing protection against infections and diseases. It must be finely balanced to defend against threats without causing excessive damage to the body's own tissues. Dysregulation of the immune system can lead to conditions such as autoimmune diseases, allergies, and immunodeficiency disorders.

Overall, the immune system is a dynamic and complex system that is crucial for maintaining health and combating disease. Its ability to distinguish between self and non-self and to remember previous encounters with pathogens is vital for effective immunity [25].

1. Cells of the immune system

1.1. Macrophages

Macrophages are phagocytes and act as scavenger monocytes are released from the bone marrow (Fig 08), circulate in the blood and enter tissues, where they mature into macrophages (Fig 09). they produce cytokines and hydrolytic enzymes; can be activated by IFN-γ cytokines; they present antigen in association with MHC II. They are sub-classified based on the tissue where they reside, such as Alveolar cells residing in lung, Kupffer cell residing in liver, microglial cells residing in brain, and osteoclast cells residing in the bone. Macrophages are resident in almost all tissues. Macrophages are relatively long-lived cells and perform several diferent functions

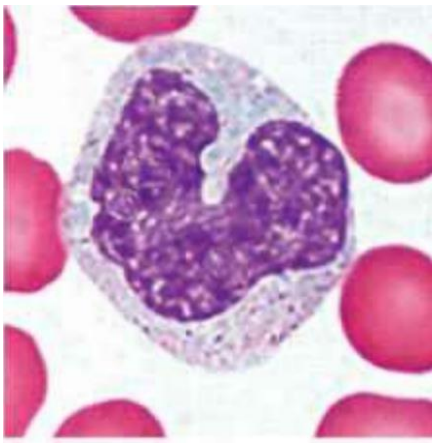

Figure 08: Light micrograph of a monocyte in a peripheral blood smear.[27]

throughout the innate immune response and the subsequent adaptive immune response. One is to engulf and kill invading microorganisms. Tis phagocytic function provides a frst defense in innate immunity. Macrophages also dispose of pathogens and infected cells targeted by an adaptive immune response. Both monocytes and macrophages are phagocytic, but most infections occur in the tissues, and so it is primarily macrophages that perform this important protective function.also is to orchestrate immune responses: they help induce infammation, which, as we shall see, is a prerequisite to a successful immune response, and they produce many infammatory mediators that activate other immune-system cells and recruit them into an immune response. Local infammation and the phagocytosis of invading bacteria can also be triggered by the activation of complement. Bacterial surfaces can activate the complement system, inducing a cascade of proteolytic reactions that coat the microbes with fragments of specifc proteins of the complement system [17].

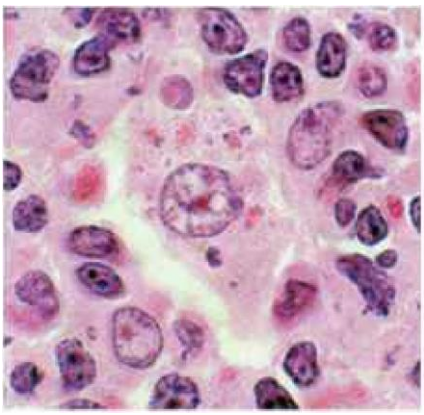

Figure 09: Light micrograph that shows macrophage [27]

1.2. Dendritic cells

Dendritic cells are large, motile, weakly phagocytic antigen presenting cells possessing several elongated pseudopodia or processes that resembles dendrite of the nerve cells thus the name dendritic cells (Fig 10). They comprise about 1% of the cells in the secondary lymphoid organs. These cells are found in different locations and are classified accordingly. These different dendritic cells have different morphology and functions but constitutively express high levels of both class II MHC molecules and the co-stimulatory B7 molecules and hence are considered more potent APC than macrophages and B cells which require prior activation to function as APC. Follicular dendritic cells however, differ in

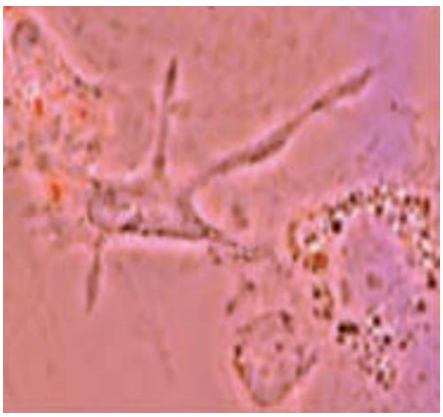

Figure 10: Light micrograph of cultured dendritic cells [27]

function from APC dendritic cells as they do not express class II MHC molecules. But these cells express high levels of membrane receptors for antibody and complement system. Binding of antigen-antibody complexes is thought to activate the B-cell activation in the lymph nodes [25].

1.3. Mast cells

Mast cells are sessile cells present throughout the body but chiefly in perivascular connective tissue, lymph nodes and mucosal epithelial tissue of the respiratory, genitourinary and digestive tracts. They are released from the bone marrow into the blood during hematopoiesis as precursor cells which get differentiated only after reaching the tissue possess large number of cytoplasmic granules containing histamine and other pharmacologically active substances and surface FC receptors with a high affinity for IgE (Fig 11). Mast cells, together with basophil cells thus play a major role in the allergic responses and protecting the internal surfaces from pathogens, including parasitic worms [17].

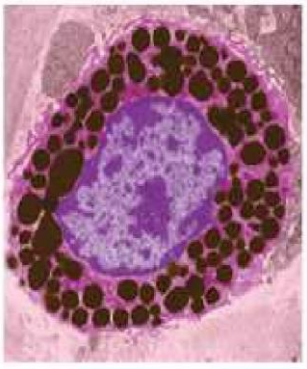

Figure 11: Light micrograph of mast cell [27]

1.4. Granulocytes

Granulocyte cells are those that contain membrane bound granules in their cytoplasm. These granules contain enzymes capable of killing microorganisms and destroying debris ingested by the process of phagocytosis. There are three types of granulocytic cells [26]:

- **Basophils**

These represent only about 1% of the leukocytes. Their nucleus is bilobed or S-shaped. They have very large, irregular basophilic granules that stain with the basic dye methylene blue. The granules contain histamine and heparin and release these contents in response to antigens and thus

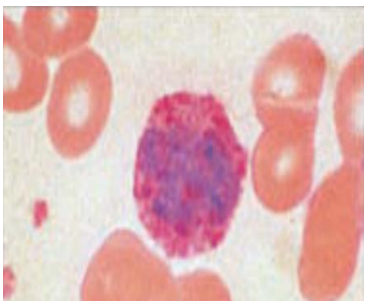

Figure 12: Light micrograph that shows basophil [27]

play a major role in allergic response. Basophils are circulating cells and possess surface FC receptors with a high affinity for IgE (fig12).

- **Eosinophils**

Eosinophils form a small proportion of peripheral blood leucocytes (1-5%) but are more prevalent in tissues. They have a bilobed nucleus and a granulated cytoplasm that stains with acid dye eosin red. They become more plentiful in circulation and in relevant tissues in allergic and parasitic diseases and their functions can be divided into effects on parasites and the inflammatory process. they are also motile and can move to the site of action. They phagocytose poorly but degranulate promptly in the presence of chemotactic factors and when membranebound IgG or IgE is cross-linked by antigen (Fig 13) . it has FC receptors for both IgG and IgE isotypes. the role of neutrophils is the intracellular digestion of microbes which are phagocytosed.

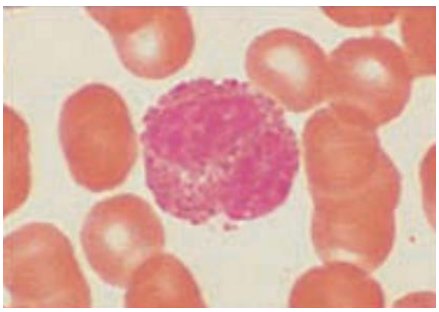

Figure 13: Light micrograph that shows eosinophil [27]

- **Neutrophils**

They are the most common of the leukocytes and represents about 55-70% of all the circulating leukocytes. They have a diameter of about 12 mm, segmented nucleus and cytoplasm packed with small specific granules that stains salmon pink colour after staining with Romanovsky type staining (Fig 14). They migrate to their tissues where they have the life span of few days. They are involved as a first line of defence against invading microorganisms and are important in inflammation and at sites of injury or wounds. During infection, their production increases, a process called leukocytosis, to arrive at a site of inflammation which is medically used as an indicator of infection. Pus that develops in sites of infection is mainly composed of dead neutrophils. Like macrophages, neutrophils are active phagocytic cells. The difference lies in the lytic enzymes and bactericidal substances that are contained in primary and secondary granules in neutrophils. Primary

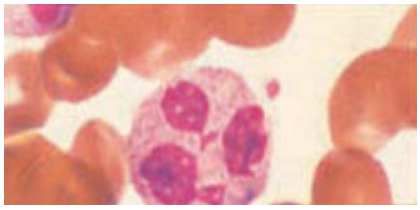

Figure 14: The light micrograph of a Wright-Giemsa–stained blood neutrophil shows the multilobed

granules are large and contain peroxidase, lysozyme, and various hydrolytic enzymes whereas secondary granules contain collagenase, lactoferrin, and lysozyme [24].

1.5. Natural killer (NK)

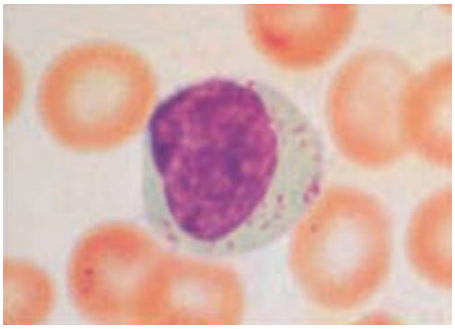

Figure 15: Light micrograph of a natural killer [27]

Cells are a small group of lymphocytes present in the peripheral blood that do not express any membrane receptors that distinguish the B- and T-cell lineages. As these cells lack antigen-binding receptors. Most members of the Natural killer cells are large, granular lymphocyte cells, constituting 5%-10% of the lymphocytes in human peripheral blood. One of the primary effector functions of the NK cells is the production of large amounts of interferon-gamma (IFN-γ) to fight of viral infections. NK cells are probably best known for their natural ability to kill tumour cells (Fig 15) . This is due to interaction between ligands on the tumor cell and a variety of receptors on the NK cell, leading to the release of the NK cell's cytotoxic granules. They function as effector cells that directly kill certain tumors such as melanomas, lymphomas and viral-infected cells. NK cells are reported to be acting in two different ways. In some cases, they make a direct membrane contact with the tumor cell in a non-specific antibody independent way. But in some, there are reports on the involvement of surface receptors (CD16) which help in recognition of the FC region of the IgG molecule and thus acting in an antibody dependent cell-mediated toxicity (ADCC). When the activating receptors and their associated signal transducing molecules are cross linked by interaction with their ligand, a signal is send in to the cell that activates a series of kinases, eventually leading to activation of the cell's cytotoxic effector function and IFN- γ. The inhibitory receptors have opposite effect. When activating and inhibitory receptors are crosslinked by their ligands on the surface of a potential target cell [25].

1.6. B lymphocytes

Are so called as they are produced and matured the bone marrow. As the B-cell matures they expresses membrane bound immunoglobulin or antibody receptors, which serves molecules of antibody bearing identical binding site for antigen. When a naïve B cell, the cell begins to divide rapidly into memory B cells and effector B cells called plasma cells (Fig 16). Memory B cells, as the name indicates, remains in the circulation for a long time and thus has a longer life-span. It continues to express the same membrane-bound antibody as the original parent naive B cell. Effector cells bring about the effect by secreting large number of antibody but they do not express the membrane bound antibody. Though the plasma cells have a very short life span of few days but they can secrete more than 2000 molecules of antibody per second. These secreted antibodies are the major effector molecule of the humoral immunity, and may be one of the five classes of antibody (IgG, IgA, IgM, IgD and IgE). All clonal progeny from a given B cell secrete antibody molecules with the same antigenic specificity [17].

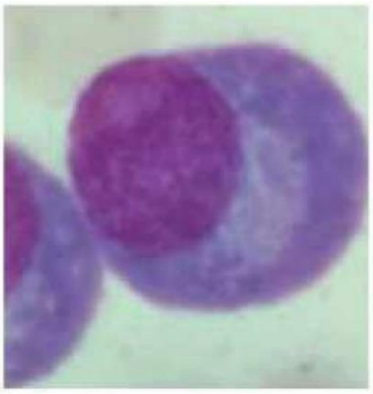

Figure 16: Light micrograph plasma cell [27]

1.7. T lymphocytes

T lymphocytes derive their name from their site of maturation in the thymus, the organ where pre-T cells, from the bone marrow, migrate to and mature in. During its maturation, it also expresses a unique antigen binding molecule on its membrane called the T cell receptor. T cells play a crucial role in the full expression of immunity by regulating antibody production, cellular immune reactions and killing of altered cells. Unlike a B-cell, a T-cell can recognize antigens only when it is processed into antigenic peptides and is bound to cell membrane proteins called major histocompatibility complex (MHC). This focusing of T-cell antigen recognition through MHC molecules is known as MHC restriction. The antigenic peptide must be displayed together with MHC molecules on the surface of antigen presenting cells, APCs (B cells, macrophages and dendritic cells) or on virusinfected cells, graft cells or cancer cells. Thus, the T-cells

are developed to eliminate such altered self-cells that pose threat to the normal functioning of the body. T cells have two welldefined subpopulations: T helper cells (TH) cells and T cytotoxic (TC) cells Like B cells, T cells subpopulations also expresses the T cell receptor specific for each population. TH cells and TC cell expresses CD4+ and CD8+ glycoproteins on their surfaces respectively. Thus the ratio of TH to TC cells in a sample can be approximated by assaying the number of CD4+ and CD8+ T cells. This ratio is approximately 2:1 in normal human peripheral blood. TH cells get activated when it encounters an antigen complexed with MHC class II molecules presented on an APC. Following its activation, T cell secretes various growth factors known as cytokines which play a central role in activation of B cells, TC cells and a variety of other cells that participate in immune response. Difference in the pattern of cytokine production elicits different activation of immune response. For example, TH1 response is designated when T cytotoxic cells and macrophages are activated and TH2 response designates the activation of B cells. TH cell maintain memory and are the principal proliferative cells responding to foreign antigen associated with self class II MHC molecules (Fig 17) [17].

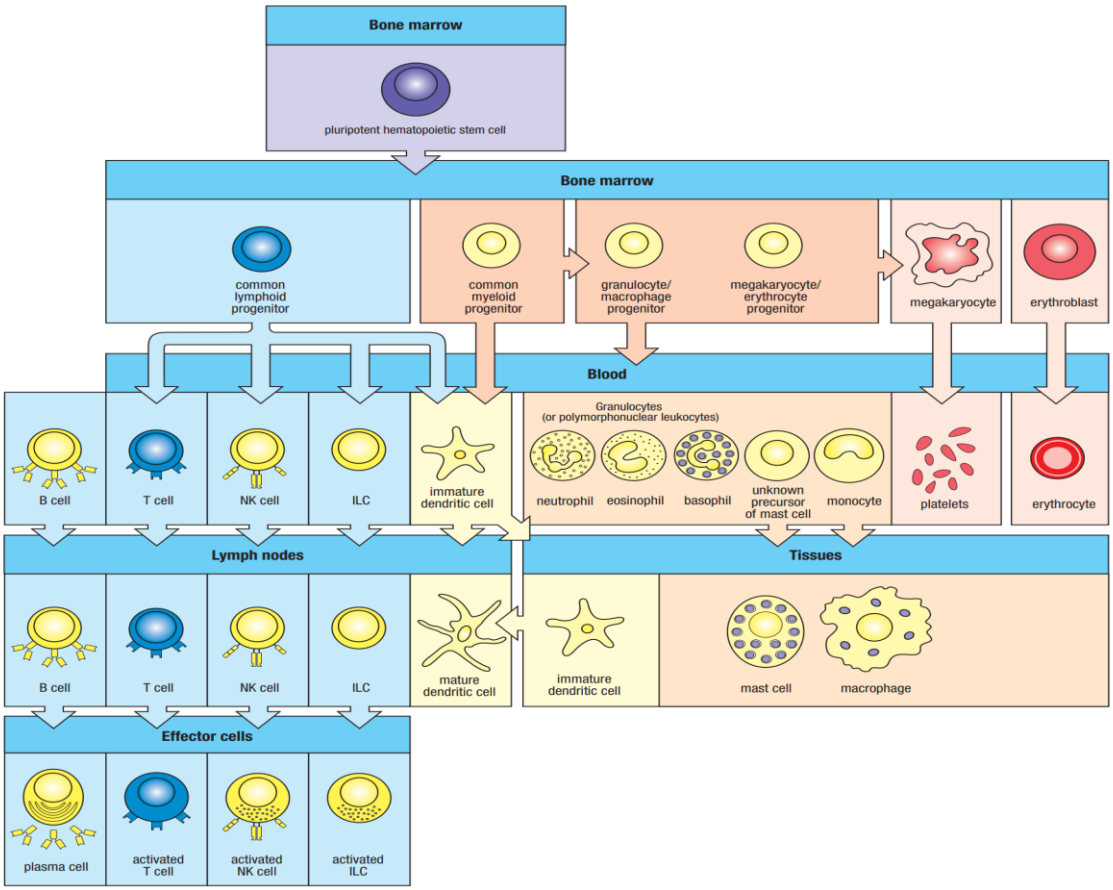

Figure 17: All the cellular elements of the blood, including the cells of the immune system, arise from pluripotent hematopoietic stem cells in the bone marrow [18]

2. Inflammatory response

2.1. Definition

Inflammation refers to the localized reaction of vascularized tissues to both internal and external triggers. Its name originates from the Latin word "inflammare," signifying a burning sensation. The primary purpose of inflammation is to contain and eradicate the initiating factor while minimizing damage to surrounding tissues. Therefore, inflammation serves as a natural physiological defense mechanism against injury. It's important to recognize that inflammation should not be viewed as a disease itself; rather, it is a beneficial process triggered in response to various forms of trauma or illness [15].

2.2. Causes of inflammation

- Infections: Often the primary instigator, infections prompt a range of inflammatory responses, spanning from mild to severe, acute to chronic. The outcome hinges greatly on the type of pathogen involved, the host's response, and inherent host characteristics.
- Tissue necrosis: Regardless of the cause of cell demise, such as ischemia, trauma, or thermal/chemical injury, tissue necrosis invariably provokes an inflammatory reaction.
- Foreign bodies: Whether due to their presence or the trauma they induce, foreign bodies incite inflammation, sometimes accompanied by associated microbes. This can occur from endogenous sources, such as urate crystal deposits seen in conditions like gout.
- Immune reactions/hypersensitivity: In cases where the normally protective immune system targets self-antigens or environmental substances, it can result in damage to the body's own tissues, as observed in autoimmune diseases and allergies. As these stimuli cannot be eradicated, the resulting inflammation often persists and proves challenging to treat, leading to significant morbidity and mortality [15].

2.3. Mechanism of inflammation

Inflammation involves a complex interplay of various mechanisms**:**

- Vasodilation: blood vessels near the affected area widen, increasing blood flow and causing redness and heat.
- Increased vascular permeability: blood vessel walls become more permeable allowing fluid, proteins, and immune cells to move into the tissue, leading to swelling.
- Chemotaxis: chemical signals attract white blood cells (leukocytes) to the site of inflammation, helping to the combat pathogens and remove debris.

- Phagocytosis: white blood cells engulf and digest foreign particles, such as bacteria or damaged cells (Fig18).
- Cytokine release: Cells release signaling molecules called cytokines, which regulate inflammation and immune responses.
- Acute-phase response: The liver produces proteins such as C-reactive protein and fibrinogen, which help to neutralize pathogens and promote tissue repair.
- Resolution and tissue repair: Inflammation eventually subsides as the immune response resolves, and tissue repair mechanisms as activates to restore normal function.[16]

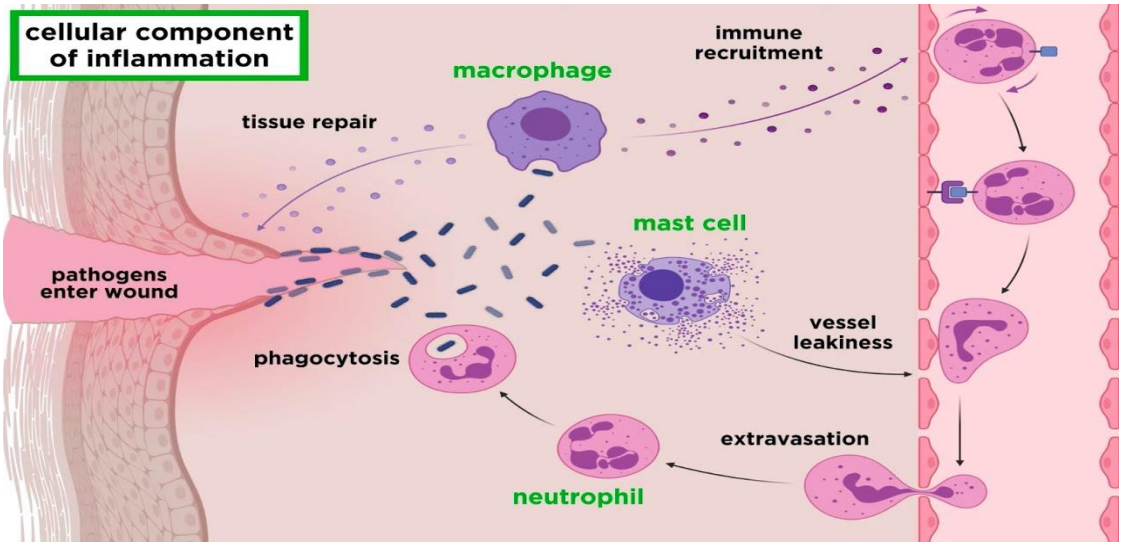

Figure 18: cellular component of inflammation [16]

3. Apoptosis

3.1. Definition of apoptosis

Apoptosis, also known as programmed cell death, is distinguished by specific morphological and biochemical features. This regulated process is crucial for normal cell turnover, immune system function, embryonic development, and cell death induced by chemicals. Dysregulation of apoptosis contributes significantly to various diseases, including neurodegenerative disorders, autoimmune conditions, and various cancers [6]. It serves as a homeostatic mechanism occurring during development and aging to maintain tissue integrity. Throughout an organism's life cycle, apoptosis plays essential

roles. For instance, in human embryo development, the separation of digits is facilitated by apoptosis occurring between the forming fingers and toes [7]. Apoptosis generates apoptotic bodies, which are promptly engulfed and eliminated by phagocytic cells, preventing damage to surrounding tissues. Furthermore, apoptosis functions as a protective mechanism, such as in immune responses or in response to cellular damage from toxins or diseases. Numerous factors and conditions can trigger apoptosis [19]. For example, in cancer treatment, chemotherapy drugs and radiation induce DNA damage, activating apoptosis through the p53-dependent pathway. Additionally, certain hormones like corticosteroids can induce apoptotic death in specific cell types without affecting others. This review aims to illuminate the mechanisms underlying programmed cell death (Fig 19) [10].

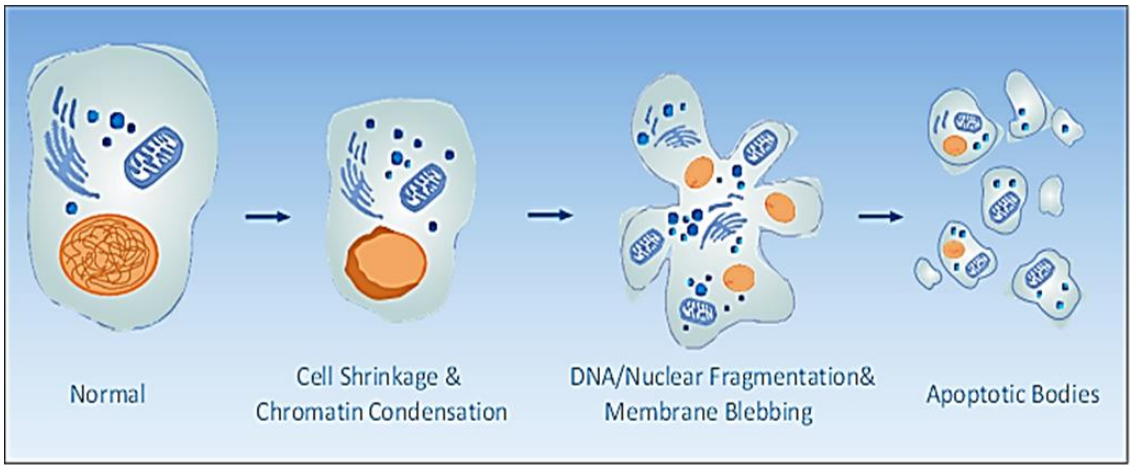

Figure 19: cytology of apoptosis [10]

3.2. Mechanisms of apoptosis

Apoptosis signaling pathways: Apoptosis is carried out through various signaling pathways, including the extrinsic or intrinsic pathways (Fig 20), and in certain instances, the perforin/granzyme B pathway, leading to the activation of the caspase cascade. Within these pathways, several protein families participate in either promoting or inhibiting caspase activation [3].

- **Extrinsic pathway:** The extrinsic pathway initiates with the binding of a ligand to one of several death receptors, which are all part of the TNF receptor superfamily.

This ligand-receptor interaction induces receptor oligomerization and recruit's adaptor proteins that contain death domains (DD), such as TRADD and FADD. Subsequently, these complexes activate pro-caspases-8 and -10. Ligands such as FASL, TNF-α, TRAIL, and TWEAK can either be anchored in the plasma membrane of adjacent cells or function as soluble cytokines [4].

- **Perforin/ Granzyme B Pathway:** In the Perforin/Granzyme B Pathway, cytotoxic cells such as cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells play a key role during immune responses against viruses and cellular transformation. These cells identify infected or transformed cells and release serine proteases known as granzymes into the cytosol of the targeted cells. Granzyme B, in particular, initiates apoptosis through two primary mechanisms: firstly, by cleaving BID, which leads to mitochondrial outer membrane permeabilization (MOMP), and secondly, by directly processing effector caspases [5].
- **Intrinsic Pathway:** The Intrinsic Pathway of caspase activation can be triggered by various factors unrelated to each other, such as DNA damage, withdrawal of growth factors, detachment from the extracellular matrix, or exposure to glucocorticoids. These stimuli initiate signaling cascades that lead to the disruption of mitochondrial integrity, the release of Cytochrome c (Cyt c), and subsequently, the activation of caspase-9 [13].

  The maintenance of mitochondrial integrity is regulated by the Bcl-2 protein family, which comprises over 20 structurally related proteins containing one to four Bcl-2 homology (BH) domains. Bcl-2 proteins are categorized into three distinct subfamilies based on the presence of BH domains and their role in either promoting or inhibiting apoptosis. The balance between pro- and anti-apoptotic members of the Bcl-2 family determines whether the cell survives an apoptotic stimulus or undergoes cell death [1].

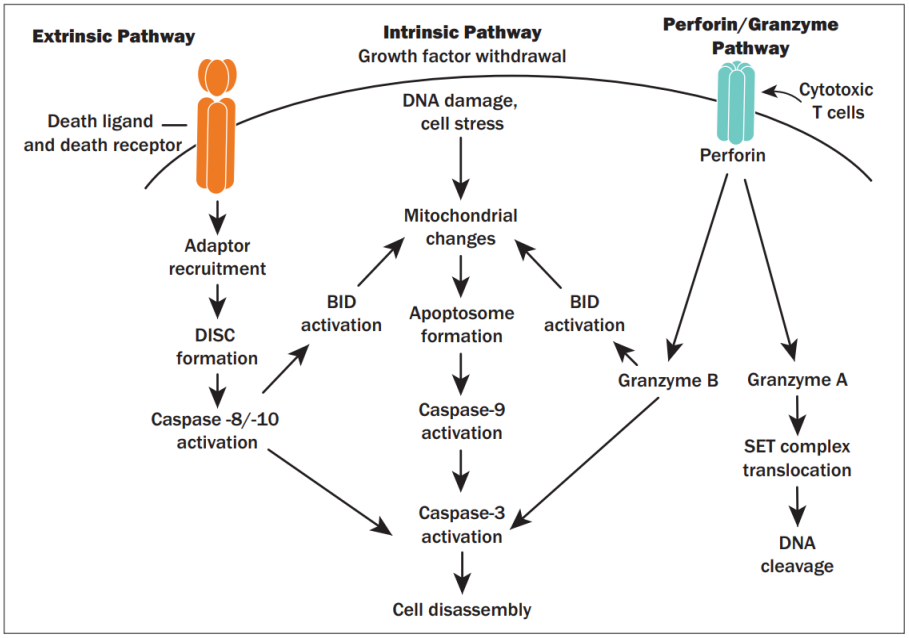

Figure 20: apoptosis pathway [3]

4. Necrosis

4.1. Definition of necrosis

Death of group of cells within a living body or the arrangement of morphological changes in mortally harmed cells those changes are irreversible due to enzymatic digestion of cell by autolysis and denaturation of protein, Necrosis happens due to:

- Cutting of local blood supply (infarction): as in thrombosis.
- Bacterial toxins: as in diphtheria toxins.
- Physical agent: as excess heat, excess cold, and mechanical trauma.
- Chemical agents: as cyanides and poisons of animals (snakes and scorpion).
- Hypersensitivity reaction: as in caseation necrosis secondary to tuberculosis [1].

4.2. Morphological evidence of necrosis

- **Cytoplasmic changes:** Swelling and vacuolization due to Rupture of mitochondria (ATP depletion) and in the endoplasmic reticulum) Injury to the lysosomal membrane followed by leakage of digestive enzymes (autolysis) and extensive damage to the cell membrane, increase Ca influx (Fig 21), denaturation of cytoplasmic proteins. So, 6 hours after necrosis the cytoplasm becomes more vacuolated, swollen homogeneous and deeply acidophilic (pink with eosin) as: Denaturized cytoplasmic proteins bind more with eosin also the Loss of ribosomes, decrease cytoplasmic basophilia.

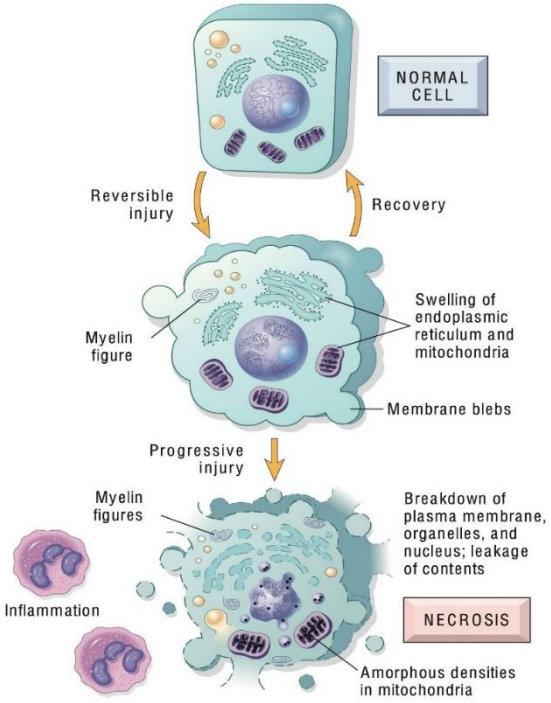

Figure 21: pathway of necrosis [7]

- **Nuclear changes:** Are the best evidence of cell necrosis
  - **Pyknosis:** Coarse, shrunken and dense nucleus
  - **Karyorrhexis:** The pyknotic nucleus breaks up into numerous small basophilic particles.
  - **Karyolysis:** In rapid necrosis the nucleus lysis without a pyknotic stage [7].

4.3. Fate of necrosis

- If small areas of necrosis, undergo lysis, phagocytosis, fibrosis
- If large areas of necrosis, capsulated by fibrous tissue, dystrophic calcification [5].

4.4. Types of necrosis

- **Coagulative necrosis: (ischemic necrosis or infraction):** Occurs in solids organs (heart kidney): due to sudden cutting of blood supply. Necrotic area is swollen, firm,

yellow, opaque. Microscopically: the outline of the tissue is preserved but all cellular details are lost (ghosts of cells are preserved).

- **Liquefactive necrosis:** Results when lysosomal enzymes are leased by the necrotic cells causing rapid liquefaction (autolysis by its own enzymes) for e.g.: brain infraction and abscess, suppurative abscess, Amoebic abscess.
- **Caseous necrosis:** It occurs in tuberculosis, cheesy in character. under Microscope we see complete loss of the affected area, become homogeneous.
- **Fibrinoid necrosis (degeneration):** Seen in autoimmune diseases (rheumatic fever, polyarthritis nodosa …etc. Fibrinoid necrosis of arterioles also occurs in malignant hypertension. Characterized by loss of normal stricter and replacement of tissue homogenous pink-staining necrotic material having strong to P stain.
- **Fat necrosis:**
  - **Enzymatic fat necrosis:** Occurs in acute pancreatitis. Pancreatic enzymes are liberated Acton TG of retro peroneal fat, glycerol with Ca, Ca soap which appears as opaque chalky white plaques in adipose tissue and omentum.
  - **Non enzymatic fat necrosis (traumatic fat necrosis):** Occurs in breast, subcutaneous tissue secondary to trauma. The necrotic fat evokes an inflammatory response characterized by foamy macrophages, neutrophils and lymphocytes (foreign body giant cell reaction). Granulation tissue forms around the necrotic lesion, fibrous tissue that may be calcified and difficult to distinguish from cancer [29].

5. Immune response via animals venom

5.1. Innate immune response

The innate immune system plays a crucial role in the initial response to animal venom. Here's how it functions:

- **Physical barriers:** The skin acts as a physical barrier, preventing venom from entering the body through superficial wounds. Mucous membranes lining the respiratory, gastrointestinal, and genitourinary tracts also serve as barriers against venom invasion.
- **Immediate response:** Upon venom injection, components of the innate immune system such as mast cells and basophils release histamines and other inflammatory mediators. This results in localized inflammation, pain, and swelling, which helps to contain the spread of venom and recruit other immune cells to the site of injury.
- **Phagocytosis:** Phagocytic cells, including neutrophils and macrophages, play a crucial role in engulfing and destroying venom components and any damaged tissue. They help to clear the site of injection of cellular debris and toxins.
- **Complement system activation:** The complement system, a group of proteins in the blood, can be activated by venom components. Activation of the complement system enhances inflammation, promotes opsonization (marking of pathogens for destruction by phagocytes), and leads to the formation of membrane attack complexes that can directly lyse pathogens.
- **Cytokine production:** Various cells of the innate immune system produce cytokines, signalling molecules that regulate the immune response. Cytokines help to coordinate the response to venom, recruit additional immune cells to the site of injury, and modulate inflammation. Overall, the innate immune system provides an immediate and nonspecific defense against animal venom, helping to limit the damage caused by venom injection and initiate the subsequent adaptive immune response, if necessary [28].

5.2. Adaptative immune response

The adaptive immune system also plays a significant role in the response to animal venom, particularly in providing a targeted and long-lasting defense against specific venom components. Here's how adaptive immunity functions against animal venom:

- **Antibody production:** Upon exposure to venom, B lymphocytes of the adaptive immune system can recognize specific venom components as foreign antigens.

B cells then produce antibodies, also known as immunoglobulins, that are tailored to bind to these venom components with high specificity. This process is known as the humoral immune response.

- **Memory response:** Following exposure to venom, some B cells differentiate into long-lived memory B cells. These memory B cells remain in the body and "remember" the specific venom components they encountered. If the individual is re-exposed to the same venom in the future, memory B cells can rapidly produce large quantities of antibodies, providing a quicker and more robust immune response.

- **T cell response:** Some venom components may also trigger a cellular immune response mediated by T lymphocytes. T cells can directly kill cells that have been infected or damaged by venom, or they can help to activate other immune cells such as macrophages and B cells. This cellular immune response is particularly important for clearing venom-infected cells and coordinating the overall immune response.

- **Antigen presentation:** Dendritic cells, macrophages, and other antigen-presenting cells play a crucial role in adaptive immunity by capturing venom components, processing them into antigenic peptides, and presenting these peptides to T cells. This process helps to initiate and regulate the adaptive immune response against venom.

- **Specificity and diversity:** The adaptive immune system's ability to generate a diverse array of antibodies and T cell receptors allows it to recognize and respond to a wide range of venom components with high specificity. This specificity enables the adaptive immune system to effectively neutralize venom and provide long-lasting protection against future exposure. Overall, the adaptive immune system enhances the body's defense against animal venom by providing a targeted and adaptable response that can effectively neutralize venom components and prevent further harm [28].

Chapter four

Molecular mechanisms of snake venom in immune cells

Introduction

The immune system is able to find and destroy pathogens, and abnormal cells, preventing the development of a variety of illnesses. Snake venoms are rich sources of bioactive proteins, many of them having enzymatic activity, and are responsible for several biological effects. This chapter delves into the snake venom effect on components of the immune system, and how some of their components can be useful for the study and development of anti-inflammatory drugs [57].

1. Modulation of Immune Cell Function

1.1. Cytokine Production

Snakes venom components can stimulate or inhibit the production of cytokines like TNF-α, IL-1β, IL-6, and IL-10, which are crucial for immune regulation. Inflammation is characterized by redness, swelling, warmth, pain and loss of function in the affected tissue, which are consequences of immune cell responses and the vascular and inflammatory events associated with infection or injury [37]. Changes at the circulatory level are related to changes in vascular permeability, leukocyte recruitment and infiltration and the release of inflammatory mediators and cytokines [38].

The immune system responds to tissue damage through the initiation of a chemical signaling cascade to repair the affected tissues. This type of chemical signal is necessary for leukocyte chemotaxis to the site of injury, where activated leukocytes are responsible for resolving the inflammatory response through the production of cytokines, chemokines and lipid mediators [37].

The toxins Bl-PLA2 and Bbill-TX from snakes B. leucurus and B. billilineata have been shown to increase the levels of proinflammatory cytokines such as IL-4, IL-6, IL-12, TNF-α and IL-1β, which are associated with hemolytic activity and leukocytosis due to neutrophilia and eosinophilia. The cytokines IL-1, IL-6 and IL-12 have multiple

functions and act synergistically to establish acute inflammation in tissues and modulate the function and differentiation of T- and B-lymphocytes [39].

Modulation of the anti-inflammatory profile has been evaluated in models of envenomation with the venom of Crotalus durissus collilineatus, Daboia russelii, C. durissus terrificus and species of Bothrops spp., and the results showed an increase in the production of IL-10 [40]. The gene expression of cytokines has been studied in response to venoms from other animals, such as that of the viper Vipera ammodytes ammodytes, and it was shown that the venom of this snake stimulates the expression of proinflammatory genes such as Il1a, Il1b, Ifna2 and Ifnb1 [41]. The same venom is capable of downregulating the production of IL-12 and IL-18, which are potent stimulators of IFN-γ release. IL-12- and IL-18- induced production of IFN-γ is important for inducing the cytotoxic activity of innate immune cells, as well as for the development and maintenance of the Th1 response [42]. Similarly, the venoms of V. ammodytes ammodytes, B. billilineata and Calloselasma rhodostoma stimulate the gene expression of IL-8 by neutrophils, which is a powerful chemoattractant of polymorphonuclear cells, CD4+ and CD8+ T-lymphocytes and NK cells [43]. Cobratoxin, cardiotoxin and neurotoxin derived from N. naja atra venom exert anti-inflammatory responses by reducing the levels of TNF-α and IL-1β and the enzymatic activities of myeloperoxidase (MPO) and iNOS [84].

1.2. Apoptosis and Necrosis

Snake venom proteins such as PLA2 and metalloproteinases can induce apoptosis (programmed cell death) or necrosis in immune cells, reducing their numbers and impairing immune responses.

- **Phospholipases A2**

Phospholipases A2 (PLA2) enzymes from Bothrops asper and Naja naja snakes catalyze the hydrolysis of phospholipids at the sn-2 position, releasing fatty acids and lysophospholipids, which disrupts cell membrane integrity, increases permeability, and

leads to cell lysis and necrosis. This hydrolysis generates lipid-derived secondary messengers, such as arachidonic acid, which can be metabolized into eicosanoids like prostaglandins, thromboxanes, and leukotrienes, all crucial in inflammation and apoptosis. Arachidonic acid, for instance, can be further processed by cyclooxygenases and lipoxygenases to produce pro-apoptotic factors. PLA2 can also target mitochondrial membranes, releasing cytochrome c and other pro-apoptotic factors, thereby triggering the intrinsic pathway of apoptosis. Consequently, PLA2 enzymes amplify inflammatory responses by producing lipid mediators that recruit and activate immune cells, contributing to tissue damage and necrosis. They also modulate immune cell function, altering macrophage and neutrophil membrane composition and signalling pathways, leading to cell death or modified immune responses.

- **Snake venom metalloproteinases**

Snake venom metalloproteinases (SVMPs) from Bothrops jararaca and Crotalus atrox degrade key components of the extracellular matrix (ECM), such as collagen, fibronectin, and laminin, disrupting the structural scaffold that supports tissues and leading to hemorrhage and necrosis. Additionally, SVMPs can directly cleave membrane proteins, compromising cell integrity and facilitating the spread of venom through tissues. SVMPs also activate apoptotic pathways by cleaving specific substrates involved in cell survival, such as matrix metalloproteinases (MMPs), which further degrade ECM components and release bioactive fragments that promote apoptosis. The biological effects of SVMPs include causing hemorrhage and edema by breaking down the ECM and basement membranes of blood vessels, leading to vascular leakage and tissue necrosis due to compromised blood supply. Furthermore, SVMPs modulate immune responses by altering cytokine production and immune cell recruitment, with the degradation of ECM components exposing cryptic sites that influence immune cell behavior, resulting in enhanced inflammation and cell death.

Synergistic Effects of PLA2 and Metalloproteinases

The combined action of PLA2 and SVMPs in snake venom can lead to extensive tissue damage. While PLA2 disrupts cell membranes and induces apoptosis through lipid signalling, SVMPs degrade the ECM and promote necrosis through vascular damage. Together, these enzymes can amplify the inflammatory response, recruiting immune cells to the site of envenomation. The resulting cytokine storm can further enhance tissue damage and immune cell death [55].

2. Signal Transduction Pathways:

2.1. NF-κB Pathway

Some snake venom components activate or inhibit the NF-κB pathway, which is critical for immune cell activation, cytokine production, and survival for example Cobratoxin from Naja naja atra decreased the levels of phosphorylated IKK-α and phosphorylated IκB-α, blocking the translocation of NF-κBp65 to the nucleus. Cobratoxin from other snakes, inhibited the NF-κB pathway by binding with high affinity to the IKK proteins in the canonical pathway; these proteins are involved in the phosphorylation and degradation of IκB.

2.2. MAPK Pathways

Mitogen-activated protein kinase pathways can be modulated by venom enzymes, influencing cell proliferation, differentiation, and inflammatory responses. This activation is coupled with a modest anti-inflammatory response that seeks to counteract the associated local and systemic effects. The initial activation of phagocytes occurs through the recognition of venom molecules by Toll-like receptors (TLRs), especially TLR2 and TLR4. Research has shown that TLR2 and TLR4 detect the Ts1 toxin from Viperidae snake venom. This β-toxin binds to TLR2 and TLR4, initiating cytokine and lipid mediator production via a MyD88-dependent pathway, which activates proinflammatory signalling pathways such as NF-κB, AP-1, and MAPKs (ERK1/2 and

p38. Also, CTX from C. durissus inhibits the production of IL-6, TNF-α, and IL-12 by interfering with the phosphorylation of NF-κBp65, ERK1/2, and MAPK p38 [54].

3. Immune Cell Recruitment and Activation

3.1. Chemotaxis

Snake venom molecules can either attract or repel immune cells like neutrophils and macrophages, influencing the site and extent of inflammation:

3.1.1. Phagocyte

The secreted phospholipase A2 (sPLA2) present in Bothrops atrox and Bothrops asper venom interacts with Toll-like receptor 2 (TLR2), initiating a cascade that leads to the production of eicosanoids such as prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) [30]. These eicosanoids act as potent chemoattractant for neutrophils, playing a crucial role in resolving the acute response at the injury site. Conversely, Calloselasma rhodostoma venom indirectly activates neutrophils via an endothelial-mediated mechanism. This venom contains phospholipases D, which hydrolyze sphingomyelin, releasing ceramides that regulate TNF-α and recruit neutrophils. Additionally, the venom enhances the production of reactive oxygen species (ROS) and the release of myeloperoxidase, which aids in developing neutrophil effector functions. IL-33 production affects aryl hydrocarbon receptors on mast cells, which respond to environmental toxins and endogenous components, triggering the production of IL-17 and ROS by mast cells. This increased IL-17 production is associated with the overexpression of CXCL5, another potent neutrophil chemoattractant, further contributing to the inflammatory response at the injury site [33]. In murine models injected intraperitoneally with venom from the snake Bothrops atrox, Toll-like receptor 2 (TLR2) stimulation promotes polymorphonuclear migration and interleukin (IL)-1β production while simultaneously inhibiting IL-6 production and mononuclear migration to the injury site. This indicates a complex immune response orchestrated by the venom, involving both pro-inflammatory and anti-inflammatory signalling pathways.

Additionally, venom components from various snake species, including Tityus serrulatus (TsV), the cardiotoxin (CTX) of Crotalus durissus terrificus [31], batroxase, and BatroxPLA2 from Bothrops atrox, as well as piratoxin-I, bothropstoxin-I, and bothropstoxin-II from Naja mocambica, have been implicated in modulating cell migration. This suggests a broader role for snake venom components in influencing immune cell behavior and trafficking, potentially contributing to the pathophysiology of envenomation [32].

3.1.2. Complement system

Bothrops atrox venom in inducing the production of complement fractions C3a and C5a. These complement fractions act as potent anaphylatoxins, which means they trigger allergic responses, such as mast cell degranulation and the chemotaxis and activation of neutrophils [36]. The complement system, a part of the immune system, can be activated by snake toxins, including those from cobras of the Naja genus and the G2 fraction of flavodoxin from Trimeresurus flavoviridis [34]. For e.g., cobras prod uce a protein similar to the C3 complement protein, called cobra venom factor (CVF) [35], which leads to the formation of an enzyme known as CVFBb. This enzyme has stable activity and serves as a C3/C5 convertase, contributing to complement activation. Similarly, the G2 fraction of flavodoxin promotes the release of C3a and the formation of the membrane attack complex, further activating the complement system [53].

3.2. Phagocytosis

Snake venom can affect phagocytosis, the process by which immune cells engulf and digest foreign particles or pathogens, in several ways:

3.2.1. Phagocytes

Snake venom employs various strategies to impair phagocytosis, the immune process responsible for engulfing and digesting foreign particles or pathogens. Direct toxicity is a prominent mechanism, whereby venom components directly interfere with the function of phagocytic immune cells like macrophages [32]. These toxins disrupt cell

membranes or interfere with crucial cellular processes, hindering the immune cells' ability to identify, engulf, and break down pathogens. Furthermore, venom toxins induce alterations in cytoskeletal proteins within immune cells, leading to changes in cell shape and impairing their phagocytic capacity. This disruption of the cytoskeleton impedes the movement and ingestion of pathogens, ultimately reducing the effectiveness of phagocytosis. These combined actions of snake venom contribute to immune evasion and exacerbate the progression of envenomation [33].

3.2.2. Mast cell

Some snakes venom toxins can activate mast cells, which are immune cells involved in the initiation and regulation of inflammatory responses. Activation of mast cells by venom toxins can lead to the release of various inflammatory mediators, such as histamine and cytokines [45], these effects can impair the ability of the immune system to effectively recognize and eliminate pathogens, contributing to the pathogenesis of envenomation [46].

4. Impact on Adaptive Immunity

4.1. B and T Lymphocytes

Snake Venom can affect the activation, proliferation, and differentiation of B and T cells, influencing antibody production and cell-mediated immune responses.

4.1.1. B lymphocyte

Crotoxin is believed to reduce IgG1 and IgG2a levels by inhibiting activation signals such as (IL-4) and disrupting the interaction between Th2 lymphocytes and B-lymphocytes mediated by CD40-CD40L [47]. IL-4 suppression is significant because it is involved in Th2 cell differentiation, MHC class II expression stimulation, and exertion of mitogenic effects on B-lymphocytes through the JAK/STAT pathway [48].

4.1.2. T lymphocyte

CTX, in addition to its effects on immunoglobulin levels, influences the depletion of lymphocytes in both peripheral blood and plasma. It achieves this by stimulating the expression of adhesion molecules like LFA-1 (integrin alpha L Betha 2) and VLA-4 (integrin Alpha4 Beta1) on lymphocytes [49]. These adhesion molecules bind to endothelial cells via their specific ligands ICAM-1 and VCAM-1, respectively. This overregulation of adhesion molecules is attributed to the action of lipoxygenase-derived mediators. Similarly, cardiotoxin III from Naja naja atra venom induces lymphocyte depletion in peripheral blood by decreasing the numbers of CD4+ and CD8+ T-lymphocytes [50]. This depletion is achieved by arresting the cell cycle in the G0/G1 phase. Genotoxicity, defined as the destructive effect is implicated in these processes, altering their integrity and triggering genetic repair on genetic material (DNA, RNA), mechanisms or apoptosis as cellular responses [51].

4.2. Antigen Presentation

Some venom components interfere with antigen-presenting cells (APCs), impacting the initiation and regulation of adaptive immune responses. For example, CTX is a major component of the venom of snakes in the genus Crotalus and stimulates receptors such as formylated peptide receptors and muscarinic receptors. The CTX in C. durissus venom comprises two subunits: CA (Crotapotin) and CB, a mildly toxic phospholipase A2 with high enzymatic activity. The CB fraction of CTX diminishes the expression of MHC type II molecules, which are essential for antigen presentation to T-lymphocytes, and reduces costimulatory molecules such as CD40, CD80 and CD86 [56].

5. Clinical Implications

5.1. Inflammatory Response

The immediate local and systemic inflammation induced by snake venom, particularly cardiotoxin (CTX), often results in severe tissue damage and systemic inflammatory response syndrome (SIRS). CTX alters glucose and glutamine

metabolism by hyperactivating enzymes such as hexokinase, glucose 6-phosphate dehydrogenase, and glutaminase in macrophages. This enzymatic hyperactivity leads to increased levels of ATP and metabolites, stimulating inflammatory and immune responses primarily through the production of NADPH. NADPH serves as a substrate for NADPH oxidase, facilitating the reduction of oxygen to superoxide anions (O2-), which are rapidly converted to hydrogen peroxide (H2O2). Furthermore, NADPH promotes the production of the anti-inflammatory cytokine IL-10. The crude venom of Crotalus durissus terrificus also induces the production of pro-inflammatory cytokines such as TNF, IL-6, and IFN-γ in mice, peaking 24-72 hours post-injection, and increases nitric oxide (NO) and H2O2 production in macrophages [52]. These toxins elevate levels of various pro-inflammatory cytokines, including TNF-α, IL-6, IL-1α, IL-1β, IL-8, and GM-CSF, contributing to systemic inflammation and an increased risk of complications

Under normal physiological conditions, NF-κB is inactive and remains bound to inhibitory proteins such as IκB (inhibitor of κB) in the cytoplasm. However, during inflammation, cytokines like tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) are released, which activate signaling pathways that lead to the phosphorylation of IκB proteins. This phosphorylation targets IκB for degradation, allowing NF-κB to be released from its inhibitory complex. Once released, NF-κB translocate to the nucleus, where it binds to specific DNA sequences and initiates the transcription of genes encoding pro-inflammatory cytokines. This process is a key mechanism by which inflammatory signals are translated into changes in gene expression, leading to the production of cytokines that drive the inflammatory response [31].

5.2. Immunosuppression

Long-term exposure to venom or administration of high doses can result in immunosuppression, heightening susceptibility to infections. For instance, cardiotoxin (CTX) treatment inhibits actin polymerization, tyrosine phosphorylation, and the activities of Rho A and Rac1 proteins, thus impeding phagocytosis for up to 14 days post-treatment.

Studies on complement activation pathways modulation have identified toxins like BjussuSP-I from Bothrops jararacussu and BpirSP27 and BpirSP41 from Bothrops pirajai as inhibitors of the classical and lectin pathways, with minor effects on the alternative pathway.

Venom toxins from various Bothrops species, notably BthTX-I and BthTX-II from B. jararacussu, and BatxLAAO from B. atrox, exhibit genotoxic effects on peripheral blood lymphocytes. This effect involves decreased microglial activation, infiltration of Th1 lymphocytes (CD4+ and IFN-γ), Th17 cells (CD4+ and IL-17), and favorable recruitment of Tregs, resulting in immune suppression. Moreover, this interaction reduces the production of IL-17A, inhibits NF-κB signaling, and impacts the production of cytokines such as IL-2, IFN-γ, IL-4, and TNF-α, thus suppressing Th1 and Th17 lymphocyte dependent inflammatory responses and reducing the risk of T cell-mediated autoimmune diseases. This inhibitory effect on the Th17 subpopulation is also observed with Naja naja atra venom and Crotalus durissus terrificus CTX, promoting the expression of Foxp3 on CD4+CD25+ and CD4+CD25− lymphocytes and suppressing IL-17 production. Additionally, these venoms induce increased DNA damage, including oxidative DNA damage, and the formation of reactive oxygen species, accompanied by reduced Glutathione levels, increased lipid peroxidation, and Phospholipase C activity, indicative of oxidative stress induction. Furthermore, they modulate gene expression patterns related to DNA damage response, oxidative stress, and apoptosis [30].

6. Therapeutic Applications as Anti-Inflammatory Drugs

CTX (cardiotoxin), a prominent component of Crotalus durissus venom, is a heterodimeric protein consisting of two subunits: CA (crotapotin) and CB. While CA serves as an accessory protein, CB is a phospholipase A2 enzyme with mild toxicity yet remarkably high enzymatic activity. Of particular interest is the CB fraction of CTX, which has demonstrated the ability to induce the production of various anti-inflammatory mediators, including interleukin-10 (IL-10), transforming growth factor-beta (TGF-β), prostaglandin E2 (PGE2), and lipoxin A4 (LXA4). These molecules play

pivotal roles in regulating the inflammatory response by suppressing pro-inflammatory cytokines, promoting immune tolerance, and facilitating tissue repair processes. Despite its venomous nature, envenomation by C. durissus tends to elicit a milder inflammatory reaction and less pain compared to envenomation by other viper species. This intriguing observation has spurred intense interest among researchers seeking to harness the potential anti-inflammatory and analgesic properties of CTX and its constituent fractions for therapeutic purposes. By unraveling the mechanisms underlying the anti-inflammatory effects of CTX, scientists aim to develop innovative treatments for a wide range of inflammatory disorders, offering new hope for patients suffering from conditions characterized by excessive inflammation and pain. Such endeavors hold promise for advancing our understanding of venom biology and paving the way for the development of novel therapeutic interventions with enhanced efficacy and safety profiles [57].

Conclusion

Conclusion

In conclusion, the intricate effects of snake venoms on immune cells, mediated through a variety of molecular mechanisms, have a profound impact on both innate and adaptive immune responses. These interactions can manifest in a wide range of clinical outcomes. On a localized level, snake venoms can cause significant tissue damage, such as necrosis, where tissue cells die, and inflammation, characterized by swelling, redness, and pain. These local effects can lead to severe complications if not promptly and effectively managed.

On a systemic level, snake venoms can modulate the immune system more broadly, influencing the production of cytokines, which are signalling molecules that regulate immune responses. This can result in an altered immune cell activity, where the normal functions of immune cells are either amplified or suppressed, leading to a dysregulated immune response. the complexity of these interactions underscores the necessity for ongoing research to better understand the mechanisms through which snake venoms affect the immune system. This knowledge is crucial for developing more effective antivenoms and therapeutic strategies, improving patient outcomes, and potentially harnessing venom components for medical applications in treating immune-related diseases.

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Abstract

Abstract:

Snake venoms are rich sources of bioactive proteins whose effects on immune cells are mediated through a variety of molecular mechanisms. These intricate interactions have a profound impact on both innate and adaptive immune responses.

Snake venoms modulate the immune system by influencing cytokine production, thereby altering immune cell activity and leading to a dysregulated immune response, inducing significant tissue damage, including necrosis and inflammation.

The aim of this study is to explore and comprehend the intricate mechanisms through which snake venoms influence immune cells, shedding light on the molecular pathways and interactions that underlie these effects.

Key words: snake venom, molecular mechanisms, immune cells.

الخلاصة:

سموم الثعابين هي مصادر غنية بالبروتينات الحيوية التي تؤثر على الخلايا المناعية من خلال مجموعة متنوعة من الآليات الجزيئية. تتمتع هذه التفاعلات المعقدة بتأثير عميق على الاستجابات المناعية الفطرية والمكتسبة.

يؤثر سم الثعابين على الجهاز المناعي من خلال التأثير على إنتاج السيتوكينات، مما يغير نشاط الخلايا المناعية ويؤدي إلى استجابة مناعية غير منظمة، مسببًا أضرارًا كبيرة للأنسجة، بما في ذلك التنخر والالتهابات.

تهدف هذه الدراسة الى استكشاف وفهم الآليات المعقدة التي تؤثر بها سموم الثعابين على الخلايا المناعية، مسلطة الضوء على المسارات الجزيئية والتفاعلات التي تكمن وراء هذه التأثيرات.

الكلمات المفتاحية: سم الثعبان، المسارات الجزيئية، الخلايا المناعية.