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THE PATHOGENESIS OF HEMOLYTIC ANEMIA: MOLECULAR AND CELLULAR MECHANISMS OF INTRAVASCULAR AND EXTRAVASCULAR HEMOLYSIS

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ABSTRACT: This article examines the pathogenesis of hemolytic anemia, with particular emphasis on the molecular and cellular mechanisms of intravascular and extravascular hemolysis. It analyzes the physiological aging and pathological destruction of erythrocytes, damage to the cell membrane and cytoskeleton, oxidative stress, activation of the complement system, and the role of immune mechanisms in the development of hemolysis. Particular attention is paid to the processes involved in intravascular hemolysis, including the destruction of erythrocytes within blood vessels, the release of free hemoglobin into plasma, its binding to haptoglobin, and the reduction in the biological availability of nitric oxide (NO). In extravascular hemolysis, the mechanisms of erythrocyte recognition by macrophages in the spleen and liver, opsonization, phagocytosis, and partial removal of the erythrocyte membrane are discussed.

KEYWORDS: hemolytic anemia, hemolysis, intravascular hemolysis, extravascular hemolysis, complement system, oxidative stress, macrophages, phagocytosis, haptoglobin, hemoglobin, bilirubin, erythropoiesis.

1. Main Mechanisms of Intravascular Hemolysis

Intravascular hemolysis is the destruction of erythrocytes within blood vessels and is characterized by disruption of membrane integrity and the release of hemoglobin into the blood plasma. One of its major mechanisms is activation of the complement system. During the terminal stage of the complement cascade, the membrane attack complex (MAC), consisting of the C5b–C9 components, is formed. MAC creates pores in the erythrocyte membrane, allowing uncontrolled movement of ions and water. Consequently, erythrocyte lysis occurs. This mechanism is particularly important in immune-mediated hemolytic processes involving strong complement activation.

Oxidative stress develops when the production of reactive oxygen species (ROS) exceeds the capacity of antioxidant defense mechanisms. ROS include superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$). These molecules oxidize hemoglobin and erythrocyte membrane proteins, leading to hemoglobin denaturation, lipid peroxidation of membrane phospholipids, and reduced erythrocyte deformability. Aggregates of denatured hemoglobin are

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known as Heinz bodies. When splenic macrophages remove damaged portions of erythrocytes through a process called pitting, the cells may acquire a characteristic bite-cell appearance.

Mechanical hemolysis occurs in patients with artificial heart valves or in microangiopathic conditions. In thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS), and disseminated intravascular coagulation (DIC), fibrin strands, platelet aggregates, or microthrombi develop within small blood vessels. As erythrocytes pass through these narrowed vessels, they undergo mechanical damage and fragmentation. As a result, schistocytes — fragmented erythrocytes — may be detected in peripheral blood.

2. Biological Effects of Free Hemoglobin

Free hemoglobin released from erythrocytes during intravascular hemolysis initially binds to haptoglobin, a plasma protein synthesized in the liver. The resulting hemoglobin–haptoglobin complex is recognized and taken up by macrophages through the CD163 receptor, where it is subsequently degraded. As hemolysis intensifies, haptoglobin stores become depleted, resulting in increased levels of free hemoglobin in plasma.

Hemoglobin that is not bound to haptoglobin may undergo oxidation and release heme, which possesses pro-oxidant properties. This can damage cellular membranes and vascular endothelium. Furthermore, free hemoglobin reacts with nitric oxide (NO), reducing its biological availability. Since NO is an important molecule responsible for vasodilation, its depletion may lead to vasoconstriction, endothelial dysfunction, and impaired microcirculation. The accumulation of hemoglobin and heme in renal tubules may also cause oxidative injury and acute kidney injury.

3. Cellular Mechanisms of Extravascular Hemolysis

Extravascular hemolysis is the removal and destruction of erythrocytes from the circulation, primarily by macrophages of the mononuclear phagocyte system in the spleen and liver. Within the red pulp of the spleen, erythrocytes pass through narrow and complex vascular pathways. Healthy, flexible erythrocytes can easily pass through these pathways and return to the circulation. However, erythrocytes with rigid membranes, spherical shapes, or antibody-coated surfaces have difficulty passing through these pathways and are retained by macrophages.

In immune-mediated hemolytic anemia, IgG antibodies directed against erythrocyte antigens bind to the erythrocyte surface. The Fc region of IgG interacts with Fcγ receptors on macrophages, leading to their activation. Consequently, macrophages may either completely phagocytose erythrocytes or remove portions of their membranes. As the membrane surface area decreases, erythrocytes become spherical, lose their deformability, and are subsequently trapped and destroyed in the spleen. Partial removal of the erythrocyte membrane is known as partial phagocytosis or membrane stripping. This mechanism plays a particularly important role in warm autoimmune hemolytic anemia.

Following phagocytosis, the erythrocyte is enclosed within a phagosome, which subsequently fuses with a lysosome to form a phagolysosome. Lysosomal enzymes degrade the cellular components.

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Globin is broken down into amino acids, whereas heme is separated into iron and porphyrin components.

The complement system also participates in extravascular hemolysis. C3b and iC3b components deposited on the erythrocyte surface are recognized by CR1 and CR3 receptors on macrophages, thereby enhancing phagocytosis. Thus, complement activation does not necessarily result in intravascular erythrocyte destruction.

4. Hemoglobin Degradation and Bilirubin Metabolism

Hemoglobin consists of two main components: globin and heme. Globin is degraded into amino acids, which are subsequently reused by the body. Iron is released from heme, while the porphyrin ring is degraded by the enzyme heme oxygenase-1 (HO-1). This process produces biliverdin, Fe^{2+}, and carbon monoxide (CO).

Biliverdin is subsequently converted into bilirubin by the enzyme biliverdin reductase. Initially formed unconjugated bilirubin is water-insoluble and is transported to the liver bound to albumin. In hepatocytes, it is conjugated with glucuronic acid by the enzyme UGT1A1, resulting in the formation of water-soluble conjugated bilirubin. It is then excreted into the intestine through bile, where intestinal microbiota contribute to the formation of urobilinogen.

As hemolysis intensifies, bilirubin production also increases. If the liver's capacity to process bilirubin becomes insufficient, the level of unconjugated bilirubin in the blood rises, potentially resulting in hemolytic jaundice.

5. Iron Metabolism and Compensatory Erythropoiesis

In hemolytic anemia, the destruction of erythrocytes does not automatically mean that the iron contained within them is lost from the body. Most of the iron is recycled. In macrophages, iron is either stored as ferritin or hemosiderin or released through ferroportin, subsequently binding to transferrin and being transported to the bone marrow. There, it is utilized for new hemoglobin synthesis. However, chronic hemolysis, hemoglobinuria, and repeated blood transfusions may alter iron balance. In particular, patients receiving frequent blood transfusions are at risk of developing secondary iron overload.

Rapid erythrocyte destruction reduces oxygen delivery to tissues. Renal hypoxia stimulates erythropoietin (EPO) production through hypoxia-inducible factor (HIF)-mediated mechanisms. EPO acts on the bone marrow, promoting the proliferation, differentiation, and maturation of erythroid cells. Consequently, reticulocyte production increases, resulting in reticulocytosis in peripheral blood. If the bone marrow cannot adequately compensate for the degree of hemolysis, the rate of erythrocyte destruction exceeds the rate of production, leading to anemia.

6. Clinical and Laboratory Findings

The main clinical manifestations of hemolytic anemia include:

- Anemia: weakness, dizziness, shortness of breath, and tachycardia.
- Jaundice: resulting from increased bilirubin production.

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- Splenomegaly: caused by erythrocyte sequestration in the spleen and increased macrophage activity.
- Hemoglobinuria: resulting from increased free hemoglobin levels, particularly in intravascular hemolysis.

The main laboratory findings include:

- Hemoglobin — decreased.
- Reticulocyte count — usually increased.
- Lactate dehydrogenase (LDH) — increased.
- Unconjugated bilirubin — increased.
- Haptoglobin — decreased, particularly in intravascular hemolysis.
- Peripheral blood smear — changes depending on the type of hemolysis.
- Direct antiglobulin test (DAT) — may be positive in immune-mediated hemolysis.

These parameters should be evaluated collectively rather than individually. For example, reticulocytosis may be absent when bone marrow function is suppressed or in the presence of iron or folate deficiency.

CONCLUSION

Hemolytic anemia is a complex pathophysiological condition that develops as a result of the premature destruction of erythrocytes before the end of their normal lifespan. In intravascular hemolysis, erythrocytes are destroyed within blood vessels, resulting in the release of hemoglobin into plasma. The complement system, oxidative stress, and mechanical damage play important roles in this process. In extravascular hemolysis, erythrocytes are primarily phagocytosed by macrophages in the spleen and liver. Both mechanisms lead to increased bilirubin production and activation of compensatory erythropoiesis in the bone marrow. However, when the rate of erythrocyte destruction exceeds the body's capacity to replace them, hemolytic anemia develops.

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