Chemistry and Biology in the Study of Blood Plasma: An Integrated Approach to Understanding Its Components and Biological Functions

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Abstract

Blood plasma is an aqueous solution rich in solutes, including electrolytes, gases, metabolites, hormones, signaling molecules, and proteins. It maintains blood and tissue fluid homeostasis, transports metabolites and drugs, and participates in biochemical regulation and signaling. Chemistry influences plasma characteristics and considerations, resulting in an overarching definition of plasma as a chemically active reaction medium. A detailed analysis of plasma properties, components, connections, and chemistry across multiple disciplines can enhance understanding of physiological mechanisms and pathology, improve disease diagnostics, and inform therapeutic strategies, including drug development, transfusions, and plasma-derived biologics.

Blood plasma acts as a transport and signal medium, providing the exchange of gases, nutrients, and waste between circulating red blood cells and tissue cells. Its bioenergetic demand is met by plasma-borne metabolic molecules supplied by peripheral tissues and organs, before returning to a central location for waste removal. The presence of biochemical catalysts in plasma can facilitate these reactions. Plasma also communicates across cells and tissues via hormones, cytokines, and other signaling molecules, stimulating or inhibiting physiological pathways.

Chapter - 1

Introduction to Blood Plasma

Blood plasma is the straw-colored extracellular fluid component of blood. It originates from serum-free blood, which is blood collected without clotting and thus without clotting products such as fibrin. The basic composition of blood plasma consists of water, electrolytes, small-molecular-weight metabolites, proteins, hormones, and other solutes. Plasma differs from serum in two principal ways: plasma contains clotting factors while serum does not, and, in a conventional setting, plasma is obtained from whole blood treated with anticoagulants, whereas serum is obtained from blood allowing to clot. There are two major features of blood plasma: a) it is a fluid compartment in which there are nutrients and waste metabolites present, b) it functions as a medium for transporting dissolved gases and other solutes to and from cellular tissue [1, 2].

Maintaining blood plasma homeostasis is essential to normal metabolic function and recovery from injuries. Abnormalities in volume status can lead to peripheral edema (when increased beyond the capacity of the perfused tissue) or shock and organ failure (when reduced). Normal capillary flow provides an exudate supply of plasma in tissues that supports cellular metabolism, deliver fuel substrates and micronutrients, crucial for reducing muscle soreness after intense exercise or therapy of burn injuries involving a large area of skin. Plasma carries metabolic waste products such as urea, creatinine, urate, and some aromatic amino acids to excretory organs. Its buffering system prevents acidemia or alkalemia from respiratory or metabolic illnesses. Other roles include the transportation of plasma proteins such

as hormones (in signaling or inputs to target organs) and drugs (in pharmacokinetics), by a) binding with different affinities, b) differing concentration in capillaries or in compartments where they bind to receptors or other molecules [3, 4, 5, 6].

Definition, origin, and basic composition

Blood plasma is the clear, yellowish fluid that forms when blood is centrifuged. It is primarily composed of water (92% by weight) and serves as a solvent, transport medium, and reaction space for the body's biomolecular constituents. Plasma contains the same basic solutes as serum, except for fibrinogen and other proteins involved in hemostasis. Thus, the two terms are often used interchangeably, although the presence of cellular components must be explicitly stated when no correction has been made for cellular mass.

Plasma accounts for about 20% of the total extracellular volume. In addition to discarding the heavy cellular components, it also concentrates smaller, soluble biomolecules for easier transport to and from the peripheral tissues. Several of these solutes are maintained at relatively high concentrations (such as glucose) and undergo rapid and continual exchanges. Others, such as ammonia and many acids and bases, are known to diffuse slowly and are not measurable as free components. There is an inevitable delay in accounting for excess or insufficient supplies, yet the mechanisms regulating their distribution and exchange are still connected to plasma composition [7, 8, 9, 10].

Biological significance in homeostasis

Successful homeostasis depends on blood plasma. In total, plasma volume remains stable, redistributing within the vasculature to ensure efficient delivery of nutrients to tissues and organs and removal of metabolic waste and carbon dioxide. The concentrations of small organic molecules, inorganic ions, and gases in plasma modulate enzyme activity and neuronal function as well as provide a considerable reserve for metabolic and signalling events. Unlike other body fluids, adaptations in the type and concentration of solutes in

blood plasma convey high-frequency information about the state of the organism to the nervous system. These changes regulate behaviour, physiological pathways, and rapid and fine-tuning feed-forward adjustments to ongoing processes. Changes in blood glucose concentration during starvation or postprandially support metabolic capacity and homeostasis. Reflexive responses employ metabolites other than glucose for signalling roles, such as Δ2-3-hydroxysuccinate, which indicates reduced mitochondrial activity [3, 11, 12, 13, 14].

Collectively, these diverse spa.-такинtpui odlhtgload pivatsnaretnig nerbo fo na aragnitni htiw srehto gnioten nrael araratgtaiwdnisiu sngolsnevitimluserpc ytilibatymoc oot nanirotsuloccaac eht os sroivaamsa sseccua sserohtiysf ffem nehw noitaicossA raeP toP no sidedda sroiva smeetttaib coidal gnitauqer si yroticurc banoisroem sothmishtetelgni koniht !yliad ssael sti ylno semag evah stseug leiruoy ym deritnoc dna Beyond integration across the nervous, alimentaire, and other body systems, blood plasma maintains osmotic and ionic homeostasis, thus affecting fluid distribution between plasma and the interstitium or lymph. Accumulation of different ions and organic solutes in plasma also alters the distribution of water between the intracellular and extracellular compartments.

Historical evolution of plasma research

The blood plasma field has seen considerable development over the past two decades. Modern plasma-related studies in the life sciences can be traced back to early observations of plasma as a structural liquid, including the discover of fibrinogen, and blood coagulation studies. Subsequent investigations identified blood as a reaction solution, while a wide range of preparative methods enabled the collection and characterization of most plasma components. Two landmark milestones radically advanced knowledge regarding the biochemical composition of plasma: Mass spectrometry allowed the detection of highly abundant and polar, low-molecular-weight metabolites, and proteomic assays enabled the analysis of hundreds of low-abundance

proteins. The integration of these new findings with established data represented a further paradigmatic shift in the field, which may be termed "plasma-omics." The functional roles of all main categories of plasma components have been extensively studied. Overall, plasma pool functions include maintenance of homeostasis, transport of nutrients and waste products, liquid volume status, centralization of endocrine signaling, and provision for immune communication and defense.

Recent research in the plasma field has gained major clinical relevance. Plasma profiling has been harnessed in both the discovery and diagnostic validation of novel biomarkers for many diseases, including cardiac diseases, diabetes, cancer, Alzheimer's disease, and COVID-19. Plasma is routinely involved in the diagnosis of many conditions not primarily affecting the circulatory system but inducing tissue damage that alters the concentration of sensitive plasma constituents. The established diagnostic potential of plasma derives from its central role as a biochemical and tissue fluid compartment collecting the products of all metabolic pathways. In addition to its use for diagnostics and biomarker discovery, plasma has been widely applied in direct therapeutic strategies, such as transfusion and exchange of plasma or specific component fractions, the production of plasma-derived pharmaceuticals (e.g., clotting factors, immunoglobulins, and cystic fibrosis medications) and pharmacological considerations (e.g., interactive protein binding) in precision medicine strategies [15, 16, 17, 18].

Modern clinical relevance

Recent research elucidating the biological significance of blood plasma has renewed interest among scientists, clinicians, and physicians in other fields. Drug tests can identify illicit substances or their metabolites in body fluids. Changes in the concentrations of specific protein groups can indicate various diseases. Thus, the composition of plasma constituents informs diagnostics, therapeutics, and the future development of personalized medicine. Metabolomics,

transcriptomics, and proteomics are central to elucidating drug mechanisms, clarifying diagnostic pathways, and predicting clinical responses.

Therapeutic monitoring and adverse effect prediction during pharmacological treatments can be guided by measuring related metabolites and their cofactors. The utility of profiling disease states with blood plasma concentrations of protein biomarkers is being addressed, and markers that integrate disease characteristics are in demand. Selected markers can distinguish chronic hepatitis B patients with and without hepatocellular carcinoma and indicate patient prognoses [19, 20, 21, 22].

Chapter - 2

Chemical Properties of Blood Plasma

Blood plasma plays a pivotal role in maintaining the activity and transport of metabolites and ions throughout the body. In addition to serving as a link between the different cellular compartments of the body, plasma provides a medium for the transport of vesicles, hormones, proteins, and other signaling molecules. These considerations lead to two distinct but complementary views of blood plasma that provide deeper insights into its chemical properties from cellular and organismal perspectives, respectively.

Plasma can be considered as a part of the chemical environment of living systems, providing a reaction medium for various solute species, including metalloenzymes that require metal cofactors. This point of view focuses on plasma composition, i.e., the concentrations of the major ions, buffers, metabolites, substrates, and coenzymes, rather than the complex mixture of specific reactants and their interactions that define an equilibrium or non-equilibrium chemical system in the laboratory or the greenhouse. All of these points of view are valid and useful, but may not be equally important for every application [23, 24, 25, 26].

pH and buffering systems

Blood plasma pH, which ranges between 7.35 and 7.45, is maintained within a narrow physiologic range by three major buffering systems: bicarbonate, phosphate, and plasma proteins. These systems help reduce the risk of acid-base disturbances arising from metabolic activities. According to the Henderson-Hasselbalch equation, the

bicarbonate buffering system is defined by the ratio of bicarbonate to carbon dioxide multiplied by a constant; deviations in this ratio can result from underlying diseases and must be evaluated as a concordant pattern.

Plasma osmolality and ionic strength reflect the presence of major ions, including sodium, potassium, calcium, magnesium, chloride, bicarbonate, and sulfate. Together, these ions generally account for more than 99% of the total ionic strength. The positive and negative charges of these ions are in balance, but their relative contributions to the overall charge vary. The predominance of sodium and chloride results in a net positive charge that influences fluid distribution between plasma and its surrounding compartments. Structural properties of water as a solvent facilitate biochemical interactions; however, some reactions are limited by low ligand concentrations or poor solubility [27, 28, 29, 30].

Osmolarity and ionic strength

Osmolarity quantifies the total concentration of solute particles in a solution, factoring in both permeant and impermeant ions. Non-permeant ions exerting osmotic effects across cell membranes regulate fluid distribution between plasma and interstitial fluid via osmosis. Na^+ , Cl^- , and HCO_3^- dominate these concentrations in blood plasma, with strong acids and bases present in drops. Osmolarity is derived from van't Hoff's law, wherein the osmotic pressure of a dilute solution equals the concentration gradient times the ideal gas constant multiplied by absolute temperature. The averaging principle acknowledges that the system tends toward electro-neutrality, prompting a slight excess of positive over negative charge in relation to the main anion.

Charge balance in plasma is governed by the principle of electroneutrality, which states that the total positive charge equals the total negative charge. Major ions contributing to this balance include sodium (Na^+), chloride (Cl^-), potassium (K^+), bicarbonate (HCO_3^-), magnesium (Mg^{2+}), and phosphate (HPO_4^{2-}). are

critical for maintaining fluid distribution between blood and tissues, regulating enzyme activities, and providing stability for lipoprotein complexes. Pathological disturbances result in hyper or hyponaatremia, influencing osmolarity and the corresponding risk of cerebral edema. Sodium concentration and plasma osmolality are commonly measured during blood tests, with deviations from reference ranges indicating dysregulation within the internal environment [31, 32, 33, 34, 35].

Solubility and chemical interactions

Aqueous solubility determines the accessibility of plasma components to cellular receptors, catalysts, and substrates. As a polar solvent, water promotes spatial separation of charged groups, facilitates hydrogen bonding, and provides a medium for electrolyte, hydrophilic metabolite, and small biomolecule transport. The preferential solvation of apolar moieties by more weakly interacting water molecules results in the clustering of these groups, thereby facilitating membrane formation and the solubilization of lipophilic compounds in biological membranes and lipoprotein complexes. The partitioning of lipophilic drugs and metabolites between water and nonpolar compartments is governed by their lipophilicity (i.e., hydrophobicity quantified by the transfer energy from water to a lipophilic phase) and directly influences their absorption, distribution, and clearance.

Plasma proteins have a diverse range of functional properties, including catalytic, structural, regulatory, and transport functions, that stem largely from differences in amino-acid sequence. The formation of complexes between major classes of proteins is common and is initiated by noncovalent specific and nonspecific interactions. Protein-protein interactions often enhance the functional efficiency of the constituent proteins and may occur within a multi-protein complex enclosing multiple substrates and/or serving multiple functions. For example, enzymes in multi-enzymatic complexes respond rapidly to increases in local substrate concentration. The concomitant presence of an enzyme and a binding partner that enhances substrate delivery, or

the presence of a serum-reactive enzyme and its substrate, usually constitutes a multi-component system that promotes reaction [36, 37, 38, 39].

Plasma as a biochemical reaction medium

Plasma constitutes a reaction milieu for biochemical conversions, yet exploring plasma-borne metabolism through such a prism remains understudied. Reactions involving plasma constituents indeed take place in the aqueous phase, yet proximity effects (through ionic strength and other interactions) differ substantially from those between reactants residing within dedicated microenvironments that ensure minimized detrimental interactions with surrounding chemical species. A view of body fluids and their constitutive components as providing catalytic environments for relevant biochemical transformations, thus directly invoking catalysis concepts, proves generally more relevant for catalyze reactions.

Reactants are often partitioned into functionally distinct compartments endowed with distinct properties to promote metabolic processing via specific biochemical pathways. Nevertheless, the body confronts both surplus delivery to these compartments and production of metabolites yielding overflow reaction that proceed extraneously to the core metabolic pathways destined to eliminate these surplus quantities. Indeed, the presence of functionally distinct blood plasma compartments serving, for example, as elaborately structured surfactant-protein dispersions is crucial for elucidating and contextualizing drug-protein interactions. Catalysts that reside primarily in the blood plasma deliver an additional layer of organizational complexity while executing their operation. Although these and other such considerations incorporate catalytic aspects, they seldom explicitly consider blood plasma itself as a biochemical reaction medium within which key blood plasma constituents operate [40, 41, 42, 43].

Chapter - 3

Plasma Proteins: Structure and Function

Albumin, globulins, and fibrinogen are the three major groups of plasma proteins; together, they constitute nearly half of the plasma protein content. Albumin is the most abundant (43% ± 9.0%), followed by immunoglobulin A (IgA), IgG, and IgM (all three classes together contribute 22% ± 3.0%); IgE is specific to allergic or anaphylactic reactions and is present in small amounts (0.1%); α1-globulin (α1-antitrypsin, α1-microglobulin, and α1-acid glycoprotein) accounts for 5% ± 1.5%); haptoglobin and ceruloplasmin together represent 5% ± 1.0%); α2-macroglobulin is present at 1% ± 0.1%); and transferrin comprises 1% ± 0.3%. β1-globulin includes α2-HS-glycoprotein and α2-Lipoprotein (lipophilin) (2% ± 0.4%); β2-globulin (β2-microglobulin, β-lipoprotein) accounts for 0.6% ± 0.1%); fibrinogen is the largest plasma protein (340 kDa), although its concentration is relatively low (3% ± 1.2%).

Plasma proteins generally contain two kinds of domains: binding sites and catalytic sites, which allow these molecules to interact with other molecules in a specific manner. The binding protein domains exhibit significant binding affinity and specificity; hence, they form stable complexes with the bound partner and regulate its metabolism. The catalytic domains provide the necessary catalytic environment for the reaction of the substrates, but they do not have high affinity or specificity towards the substrates. Plasma proteins are responsible for transport (mainly by serum albumin) and also play a role in maintaining colloidal osmotic pressure. They include immune components that function in defense against infections and wounds, whereas others that participate in the coagulation cascade are prerequisites for hemostasis.

Changes in the plasma concentration of these proteins can serve as indicators of injury or disease, with diagnostic tests commonly utilized for the determination of their concentrations [44, 45, 46, 47].

Classification: Albumin, globulins, fibrinogen

The plasma protein family is made up of albumin, globulins and fibrinogen. The relative abundance of various plasma proteins is consistent although some appear in blood selected from specific tissues or during different pathological states. Albumin is synthesized almost exclusively in the liver and is the most abundant protein in plasma, making up approximately 60% of total protein. In mammals, plasma albumin has a single polypeptide chain of 585 residues and two identical disulfide-linked domains. In physiological association with anionic lipids such as phospholipids and long-chain fatty acids, albumin is responsible for the copious transport of these predominant lipids and binding of many ligands. The protein crystallizes readily owing to its stability and forms one of the few protein crystals containing a ligand. Other plasma proteins include α-, β-, and γ-globulins. The α-class consists primarily of α1-antitrypsin and α2-macroglobulin, which have a role in antiproteolytic activity and the binding of proteinases and lipoprotein lipase. Ceruloplasmin, the primary copper protein in blood, is part of this group. The primary components of the β-class are transferrin and β-lipoproteins, responsible for the transport of iron and lipids, respectively. The γ-class contains antibodies (immunoglobulins), which are key players in the specific immune response. Fibrinogen is the principal coagulation factor, involved in cross-linking during clot formation [48, 49, 50, 51].

Structural characteristics of major proteins

The three major proteins in human blood plasma have unique structures that can be briefly summarized as follows. Albumin denotes the most abundant plasma protein in all mammals, comprising a single polypeptide chain of 585 amino acids. Its structure consists of 17 domains and considerable internal disulfide bonding, thereby contributing to its high stability. Further, many highly variable binding

sites are located on its surface, which endows albumin with the capacity to serve as a general transport protein in circulation. The characteristic structure of immunoglobulins consists of two identical heavy chains and two identical light chains; these chains are organized into domains and linked by disulfide bridges. The complement system comprises many proteins categorized into three groups on the basis of structural similarities but with varied functions some function as enzymes, others as cofactors, and a few possess receptor- or binding-protein-like properties. Substrates for these enzymes are other proteins in plasma and tissues; such substrates are prepared for further cleavage by other members of the complement system.

The biological roles of these proteins in plasma circulation are equally diverse. Central functions of immunoglobulins include the recognition of antigens, contribution to the innate immune response through complement activation, and inhibition of bacterial replication via opsonization and neutralization. In contrast, the complement system is essential for innate immunity and functions mainly by marking pathogens for destruction. It additionally provides distinct links between humoral and cellular immunity. The various classes of complement proteins and their activation fragments intervene in multiple processes, including opsonization, chemokine activity, direct pathogen destruction, and enhancement of antibody responses [52, 53, 54, 55].

Functional roles in circulation

Constituents of blood plasma exert crucial functions in circulation, such as transport and distribution of lipids and hormones, maintenance of colloidal osmotic pressure, response to infection and injury, and preservation of homeostasis. Specific alterations in plasma protein concentrations are related to various diseases, and plasma protein levels are frequently analyzed as diagnostic indicators.

Multiple types of transport proteins regulate the delivery of nutrient lipids, cholesterol, and fat-soluble vitamins to tissues in the form of lipoprotein particles (chylomicrons, VLDL, LDL, and HDL).

Specific plasma proteins, such as albumin, provide a high surface area and a multitude of hydrophilic binding sites for small-molecule solutes, including electrolytes; abnormally low concentrations result in edema (fluid accumulation in tissues). The adaptive immune response relies on antibodies that specifically recognize and neutralize pathogens; pathogen detection triggers a cascade of immune responses that leads to inflammation, natural immune activation, and finally repair. Specific plasma-based markers of these responses are commonly used in clinical diagnostics.

Biomarkers of disease often arise from the detection and analysis of low-molecular-weight metabolites. The pool of metabolites in blood plasma reflects ongoing cellular processes and can indicate the status of individual tissues as well as the behavior of avascular chambers. Many metabolites are monitored in clinical laboratories; abnormal concentration changes can thus support disease diagnosis and predict patient prognosis [56, 57, 58, 59].

Clinical alterations and diagnostic value

Concentration changes in most plasma constituents are associated with distinct physiological derangements and disease states. Given the large number of potential clinical manifestations, the analytical sensitivity of current plasma measurement techniques, and the widespread availability of assays for individual analytes, altered plasma metabolite patterns can serve as useful diagnostic indicators. Important examples include the use of altered concentrations of plasma glucose or urea in diabetes or renal diseases, respectively.

The potential for specific metabolite patterns to improve diagnostic accuracy is increasingly recognized. For example, the cumulative alterations in 15 plasma metabolites of the amino-acid, fatty-acid, and lipid metabolic pathways detected by high-throughput metabolomics analysis in patients with sepsis either correlating to severity (elevated levels of glucose, homovanillic acid, benzenepropanol-3-alcohol, N,N,N-trimethylglycine, cystathionine, and palmitic acids) or predicting clinical deterioration (increasing concentrations of

cystathionine, glutamine, tryptophan, and hypoxanthine) were recently shown to help assess sepsis severity. Furthermore, the combination of three molecular mechanisms of tumoral neuroendocrine differentiation in plasma samples of patients with advanced-stage peripheral neuroectodermal tumors proved to predict poor outcome and survival [60, 61, 62, 63].

Chapter - 4

Lipids and Lipoproteins in Plasma

Lipids in plasma include free fatty acids, triglycerides, phospholipids, and cholesterol esters. Free fatty acids are present in low micromolar concentrations as products of lipolysis and are mainly bound to albumin. The bulk of plasma lipids is represented by triglycerides, which are found in lipoprotein particles. Lipoproteins consist of a polar lipid monolayer surrounding a nonpolar core of triglycerides and/or cholesteryl esters, with integral and peripheral apolipoproteins. They are classified by particle density into High-Density Lipoproteins (HDL), Low-Density Lipoproteins (LDL), and Very-Low-Density Lipoproteins (VLDL). HDL particles, rich in apolipoproteins A, are associated with cholesterol uptake from tissues and fecal transport. ApoE-rich lipoproteins facilitate chylomicron and VLDL remnant uptake, while apoB is required for hepatocellular VLDL release and tissue lipoprotein catabolism. Lipoprotein metabolism involves progressive remodeling mediated by lipase activities and endocytosis, with fatty acids, cholesterol, or both delivered to extrahepatic tissues and the liver.

Disorders of lipid metabolism cause dyslipidemias associated with accelerated atherosclerosis and cardiovascular events. Laboratory determination of total cholesterol, triglyceride, and lipoprotein levels is routinely performed to assess dyslipidemia risk and therapeutic efficacy.

Types of lipids in plasma

Plasma lipids are classified as free fatty acids, triglycerides,

phospholipids, cholesteryl esters, and lipoproteins. Free fatty acids (FAs) are present at nanomolar to low micromolar concentrations, primarily as a result of lipolysis. The majority of free fatty acids in plasma bind to albumin, with a fraction transported unbound. Triglycerides are the most abundant lipids in plasma, occurring primarily in the core of lipoprotein particles. Cholesterol is present as free cholesterol in cell membranes and esterified with fatty acids in lipid droplets and lipoprotein particles. Phospholipids are present mainly in lipoproteins [64, 65, 66, 67].

Types of plasma lipids

Several classes of lipid molecules circulate in human plasma. These include free fatty acids, triglycerides, phospholipids, cholesterol and cholesterol esters. Free fatty acids, present at millimolar concentrations, are predominantly derived from the action of lipases on adipose tissue triglycerides. Triglycerides comprise the major lipid form in the body and are endowed with a central role in energy storage, as well as serving as a transport form for insoluble fatty acids. In addition to supplying fuel during periods of increased metabolic demand, fatty acids serve as precursors for local-acting anabolic and catabolic signaling molecules such as prostaglandins and leukotrienes. Fatty acids are also important constituents of membrane phospholipids and cholesteryl esters, the latter also playing a part in lipoprotein metabolism and storage of lipophilic vitamins. Plasma triglycerides and free fatty acids must therefore be monitored in dyslipidaemia-associated cardiovascular risk assessments.

Phospholipids are prevalent components of cell membranes and are also found in large quantities in the pulmonary surfactant system. Considerably smaller amounts of cholesterol circulate in plasma as well as in other body fluids. Cholesterol does not serve as a fuel source and is invariably transported in esterified form within cholesteryl esters, the mass of these esters being especially high in very low density lipoproteins. Classic non-esterified cholesterol mediates membrane fluidity, viability, and integrity, while simultaneously offering

anchoring points for the assembly of lipid rafts. However, excessive levels of oxidized cholesterol are known to be involved in the atherogenic processes associated with cardiovascular disease. Plasma cholesterol status is therefore monitored in both routine medical assessments and cardiovascular risk analyses [64, 68, 69, 70].

Structure of lipoprotein particles (HDL, LDL, VLDL)

All plasma lipoproteins have a spherical structure with a core made predominantly of neutral lipids that are largely hydrophobic and surrounded by a phospholipid monolayer composed mainly of a glycerolipid bilayer and free cholesterol. The surface layer further contains apolipoproteins, which assume a variety of functions including structural roles and are specifically recognized by metabolizing enzymes and tissue receptors. Because of their precise role in lipid metabolism, apolipoproteins can be grouped according to source and action in a given metabolic pathway. Apolipoprotein A-I, A-II, and A-IV belong to the HDL particles while Apo B-48 and B-100 are richly concentrated in the chylomicrons and LDL, respectively. The HDL particles transport lipids from the peripheral tissues back to the liver or carry excess cholesterol to its excretion.

The metabolic processes controlling the levels of the plasma lipoproteins are extremely complex and the lipoproteins themselves are in constant evolution, showing great plasticity. Upon release from their main site of synthesis they are continuously remodeled by a number of enzymes acting in the plasma. The plasma lipoproteins are closely related to certain cardiovascular diseases and changes in the profiles of certain specific fractions or in the activity of the main classes of enzymes involved in lipoprotein remodeling have been used as markers of risk [71, 72, 73, 74].

Metabolic pathways and transport

Blood plasma serves as an important transport medium. Oxygen is transported either dissolved in plasma or associated with hemoglobin in red blood cells; carbon dioxide is carried as dissolved gas,

bicarbonate anion or carbamino compounds; and nitrogen is present in trace amounts, primarily as free gas. The metabolism of carbohydrates and amino acids produces metabolites that are transported by plasma; glucose, lactate, pyruvate and other glycolytic intermediates; citric acid cycle intermediates; diketone bodies; as well as urea and ammonia are all found in plasma. The transport of fatty acids and lipophilic vitamins poses unique challenges because water-soluble carriers are needed. Special plasma proteins such as transferrin (iron transport), albumin (junction point of multiple transport modes) and lipoproteins (complexes of lipids and proteins) facilitate lipid transport.

Drugs can become bound to plasma proteins; only the unbound fraction is biologically active. Non-polar drugs are usually bound by albumin. Binding can alter the pharmacokinetics and pharmacodynamics of the drug, affecting its action and dosage, and affecting the therapeutic window. When a drug is administered with another drug that binds to the same site on a plasma protein, it can cause unwanted side effects by displacing the original drug [75, 40, 76, 77].

Lipid-related disorders and biomarkers

Elevated circulating lipid concentrations, particularly low-density lipoprotein cholesterol, triglycerides, and lipoprotein(a), are closely linked to an increased risk of atherogenic cardiovascular disease. Atherosclerosis is implicated in a large proportion of cardiovascular events, and therefore requires careful prevention and treatment, including management of LDL cholesterol and / or triglycerides. In certain individuals, measurement of apolipoprotein B and lipoprotein(a) provide additional diagnostic information, while persistently elevated triglycerides represent a risk not only for cardiovascular disease, but also pancreatitis. Disorders of lipid metabolism also include dysregulation of high-density lipoprotein, elevated non-esterified fatty acids and secondary alterations related to type 2 diabetes.

Detailed lipid profiling, aided by the development of novel mass-spectrometry-based methods with enhanced specificity and sensitivity,

in addition to the use of lipidomic approaches, further elucidates and refines associations with disease risk. New biomarkers of dyslipidemia associated with the presence of plaque and coronary artery disease have also been proposed, including lipid transfer protein measurements. However, the precise relationships are complex and still under investigation [78, 79, 80].

Chapter - 5

Carbohydrates and Low-Molecular-Weight Metabolites

Blood glucose homeostasis and the regulation of glycolysis are critical aspects of mammalian carbohydrate metabolism. Glycolytic intermediates and other central metabolites perfusing in blood plasma reflect their plasma concentrations at any given moment, while low-molecular-weight metabolites produced by the total body via various routes follow peripheral blood circulation during the course of metabolism and excretion. In the plasma milieu, these glycolytic intermediates, TCA metabolites, amino-acid derived metabolites, nucleotide-derived metabolites, and ketone bodies exist at low concentrations relative to blood plasma lipids, proteins, steroid hormones, and other major constituents.

Plasma glucose concentration is tightly regulated through a complex network of hormones to ensure a constant supply of glucose during tireless processes of glycolysis, gluconeogenesis, and glycogenolysis. Glycolysis is predominantly regulated by the most energetically irreversible and highly regulated reactions catalyzed by glucokinase, phosphofructokinase-1, and pyruvate kinase. Clinical plasma glucose measurement is used in the diagnosis of diabetes mellitus and for screening of some minor endocrine disorders. Concentration of plasma glucose is also monitored during the diagnosis, classification, and therapeutic management of diabetes mellitus [81, 82, 83, 84].

Glucose regulation and metabolism

Blood plasma glucose levels are tightly regulated within a narrow

range, usually between 3.9 and 5.6 mM, for typ 2 diabetes (or fasting) and below 7.8 mM two hours after a meal for type 2 diabetes (or 2 h postprandial). Outside this range, glucose undergoes compartmentalization when metabolized. Simple surges in blood glucose concentrations may result within the pathogenic range. Chronic elevation and even shallow violations of normal levels can eventually lead to diabetes, a common metabolic disorder with grave complications. Glucose serves as the main energy source in vivo and is also essential for the synthesis of many bioactive compounds as a precursor or component. Catabolism provides heat for thermoregulation, whereas biosynthesis contributes to thermoregulation and fat deposition in small iguanas. Because of its central status in metabolism, the concentrations of glucose and its anabolism and catabolism throughout the glycolytic and TCA pathways are tightly regulated, and dysregulation often hints at future development of type 2 diabetes, including abnormal glucose tolerance and insulin secretion.

Glycolysis serves not only as catabolism responsible for glucose, galactose, and fructose but also as a key central metabolic pathway, and the concentrations of pancreatis functionally important noncarbohydrate metabolites, such as α-ketoglutarate and N-acetyl aspartate, including both end products and substrates of glycolysis, spatially follow those of plasma lactate and pyruvate. These noncarbohydrate metabolites correlate between glycolysis and the reversed gluconeogenesis in the daily and seasonal glucose homeostasis of animal physiology. As epigenetic bits and pieces of ultrastructure, changes in the current concentrations may respond to stress in a forward direction, approaching a boundary concentration along the daily and seasonal cycles [5, 85, 86, 87].

Glycolytic and TCA metabolites

The plasma metabolome encompasses an array of glycolytic and tricarboxylic acid cycle intermediates, along with non-carbohydrate constituents. Glucose concentration in plasma is tightly regulated, with

the actions of glucagon and insulin determining flux through glycolysis in peripheral tissues. Disorders of glucose metabolism occur in diverse clinical scenarios, with diabetes mellitus as the archetype. Plasma levels of glycolytic intermediates are governed by the activity of active and inactive glycolytic enzymes. Dysregulation is invariably associated with substrate imbalance in other metabolic networks.

Considerable scientific interest has been directed toward the link between plasma metabolite levels and disease states. Early reflections on the subject suggested that the concentrations of key TCA cycle intermediates are indicative of both mitochondrial function and metabolic state. It is hardly surprising, therefore, that aberrations in the levels of these small-molecule metabolites have been implicated in the progression of neoplastic, neurodegenerative, and cardiovascular diseases, among others. Non-carbohydrate metabolites products and by-products of amino acid, purine, and pyrimidine metabolism as well as of ketogenesis also exert pleiotropic effects on health and disease.

Results from laboratory testing and the clinical interpretation of grades of glycolytic and TCA metabolites are reviewed. While glucose is routinely analyzed by the clinical laboratory, quantitative determination and interpretation of plasma concentrations of other glycolytic and TCA cycle intermediates have thus far attracted scant attention. Nevertheless, testing these compounds reveals patterns that provide critical insight into metabolic derangement across a spectrum of conditions [88, 89, 90, 91].

Non-carbohydrate metabolites

Regulation of blood glucose homeostasis involves the action of hormones and numerous organs; dysregulation can lead to renal failure, coma, and death. The association of hyperglycemia with vascular complications and microRNA profiling in plasma/serum indicate additional clinical applications. A plethora of small molecules are detected in plasma/serum samples, including metabolites of tri-, tetra-, penta-, and hexose sugars, sugar alcohols, amino acids, carboxylic

acids, biogenic amines, nucleosides, lactate, and ketone bodies. Glycolytic and TCA cycle metabolites can also be identified.

Aside from the metabolites of carbohydrates, those of other classes of biomolecules also circulate in human plasma/serum. Acetyl-CoA and ketone bodies such as 3-hydroxybutyrate likely represent a source of fuel for other organs, especially during fasting. N-acetylneuraminic acid, erythritol, 2-deoxy-d-glucose, and ribonucleosides are of potential interest. Cystathionine, cystine, and homovanillic acid represent metabolites of amino acids and neurotransmitters. Heterocycles appear to be associated with nucleic acids, while carotenoid-based compounds likely reflect alterations in other lipophilic substances. A variety of organic acids have also been detected, some possibly derived from panel diseases. Analytical challenges involving new technologies are now resulting in deeper exploration of human plasma/serum composition at all levels of atomic resolution [92, 93, 94, 95].

Laboratory tests and diagnostic use

Blood plasma is rich in numerous molecules; therefore, it contains many diagnostic biomarkers. These biomarkers can be divided into different classes, and for many classes, there exist numerous specific molecules. The first class is proteins. Although on a protein scale, plasma contains similar amounts of many proteins, their diagnostic use is often related to their relatively low concentrations compared to other classes of proteins, e.g. enzymes or antibodies. The second class is involved in central carbon metabolism. Due to the importance of glucose homeostasis, its metabolism is tightly controlled, and abnormal concentration in plasma indicates the failure of the control system. Other diagnostic metabolic markers, such as metabolite levels from glycolysis, the TCA cycle, aromatic amino acid metabolism, and fatty acid metabolism, are closely related to different diseases. The third class is derived from nucleic acid metabolism. Circulating cell-free DNA, RNA, and small RNAs also exist in blood plasma and have been implicated in various biological processes. The focus for these non-

carbohydrate metabolites is mainly on those that are different to those found in glucose or TCA cycle. The fourth class is a group of non-protein signaling organisers, of which peptide hormones and their receptors form the classical focus of activation. Other secreted signaling molecules, such as steroid hormones, adrenal metabolites, and cytokines, have all been shown to influence many physiological processes.

Many laboratory tests of plasma are continuously performed for clinical monitoring and diagnosis; for example, biochemical tests for the main metabolic substrates and mediators, complete blood count or hematology panels, and tests for specific proteins such as C-reactive protein in relation to inflammation and clotting factor testing as a part of haemostatic assessment. With the presence of an increasing number of baso- and potentially pathologic biomarkers (genetic, cytosolic, secreted, epigenetic, etc.) described in recent times, there is an effort to widen the use of both specific biomarkers and panels of drawn-plasma assays to enhance disease diagnosis, prognosis, and monitoring of disease progression and treatment [96, 97, 98, 99].

Chapter - 6

Hormones and Signaling Molecules in Plasma

A diverse group of signaling molecules in plasma generates a wealth of information throughout the body. Chemical messengers enable regulatory networks that convey information between organs and tissues across considerable distances and initiate rapid responses to maintain homeostasis. Plasma hormones include: peptide and protein hormones such as insulin, glucagon, and anterior pituitary hormones; steroid hormones like cortisol, estrogen, and testosterone; and hormones derived from fatty acids, including prostaglandins and some eicosanoids. Hormones become released from their production sites into plasma and travel to their target tissues for action; target cells express cognate receptors that recognize and bind specific hormones to initiate defined signaling processes. Hormonal activity can occur as a rapid physiological response or as a more gradual change in physiology.

Hormones regulate a multitude of physiological functions bones, meat, fat, and milk synthesis; growth and development of tissues; maintenance of energy balance and requirements; production and maintenance of red blood cells; and maintenance responses (e.g., heat and cold) by eliciting target tissue responses. Peptide/protein hormones have a relatively short half-life in plasma (min to h). Hormones may exert effects through distinct canonical pathways or in concert with each other. Cytokines and chemokines, crucial for the recognition and regulation of immune responses, play a role not only in immune system signaling but also in the regulation of numerous physiological processes in other tissues and organs. These small proteins initiate

signaling cascades through specific cognate receptors in target cells and contribute to diverse actions, ranging from local tissue responses to systemic control such as fever [100, 101, 102, 103].

Peptide and protein hormones

Hormones are bioactive molecules acting as messengers carrying information in the body through the bloodstream, binding to specific receptors on target tissues to trigger responses. Hormones belonging to the peptide hormone family are generally synthesized in the endoplasmic reticulum and packaged in vesicles in the Golgi apparatus for subsequent storage and release. They are water-soluble and have short half-lives. Since they cannot cross the plasma membrane of target cells, they bind to specific receptors located on the outer plasma membrane such as G-protein-coupled receptors or tyrosine kinase-associated receptors, transducing the signal across the plasma membrane through signal transduction cascades that modulate gene expression and protein activity.

Besides peptide and protein hormones, other molecules also function as hormones in the body. The steroid hormones are lipid-derived molecules that affect the target cells via interaction with intracellular receptors. They are not stored in cells, but are released into circulation and taken up by the target cells. Hormones derived from arachidonic acid such as prostaglandins and leukotrienes are secreted by all cells that synthesize them, and exert local action in neighbouring cells of the same tissue. Lastly, several neurotransmitters and neuropeptides act as hormones in the products of the adrenal medulla, some neurotransmitters such as epinephrine and norepinephrine also act as hormones by changing the activity of many cells in the body. The levels of these molecules in plasma simultaneously respond to the physiological demand of the body, and the diagnostic indication of their imbalances.

Key features of signaling cascade networks during intercellular communication are easily described by concentrating on the response system in target cells and studying the activation of the receptors

responding to stimulation signals. Cytokines released by activated macrophages can communicate with other immune cells through cascading sequences, leading to the activation of naive T lymphocytes and providing the mechanism for the linkage between innate and adaptive immunity. The presence of the cytokines during the T-cell activation creates a system regulating the response of the adaptive immune system [104, 105, 106, 107].

Steroid hormones and fatty acid derivatives

Two major groups of hormones are synthesized from cholesterol: steroids and the so-called eicosanoids, which arise from polyunsaturated fatty acids. Although steroid hormones have a very low plasma concentration, their half-lives last several hours or even days (except for aldosterone, which is rapidly degraded) because they bind to high-affinity, low-capacity plasma proteins. They are maintained at stable concentrations for long periods, thus altering gene expression for cell differentiation and regulation of hydromineral homeostasis. Other hormones of lower plasma concentration, such as thyroid hormones, have similar structural characteristics and physiological actions.

Many tissues produce hormones and signaling molecules derived from arachidonic acid, such as prostaglandins, thromboxanes, and leukotrienes. These eicosanoids act locally in autocrine and paracrine manners and are generally rapidly degraded to avoid compound buildup and disarray of the complex vascular and cellular objectives of the blood. Eicosanoid half-lives are short less than 1 h for prostaglandins and vary according to their chemical structure and the action type. Transduction of their effects in target cells occurs through G protein-coupled receptors and modulates a great variety of biological functions, from hemostasis to labor [108, 109, 110, 111].

Signal transduction mechanisms

Cells communicate information using a myriad of signaling molecules, including hormones and other regulatory factors present in

blood plasma. Once produced and released into the circulation, these chemical messengers elicit specific physiological responses by interacting with a selection of target cells over considerable distances. Upon binding to membrane-bound receptors, they activate intracellular signal transduction pathways that modulate gene-expression patterns and trigger distinct cellular responses. Signaling pathways are usually classified into three broad classes according to their speed and range: local signaling in which cells send signals only to adjacent ones; neuronal signaling which allows transmission of signals at a high speed along axons connecting separate tissues; and endocrine signaling which enables communication across the entire body due to the circulation of hormones, cytokines, and other signaling molecules in blood plasma.

These signaling pathways can be classified as canonical and non-canonical according to the presence of multiple signal transduction routes engaging the same target genes. Such cross-talk among pathways enables the fine tuning of cellular responses. Many endocrine disorders such as diabetes mellitus, adrenal insufficiency, and thyroid disease alter the concentrations of plasma hormonal regulators and consequently the physiology of target tissues. A wide variety of different diagnostic indicators corresponding to endocrine disorders have been identified.

Endocrine disorders and diagnostic indicators

Endocrine disorders cause abnormal levels of circulating hormones. Hyper- or hyposecretion of peptide and protein hormones can be evaluated by measuring plasma levels. Clinical interpretation depends on hormone function and activity in target tissues. Diagnosing adrenal dysfunction often requires assessing multiple hormones and their precursors. For example, investigation of suspected primary adrenocortical insufficiency combines measurement of ACTH and cortisol. Increased ACTH with decreased cortisol suggests primary disease, while decreased ACTH with decreased cortisol is consistent with secondary adrenal insufficiency. Disorders of steroid hormones

can be evaluated by measuring testosterone, estrogens, or progestagens. The activity of these hormones is modified by sulfate and glucuronide conjugation, aromatization, and especially for testosterone conversion to dihydrotestosterone.

Plasma concentrations of thyroid hormones are clinically important indicators of thyroid dysfunction. Most circulating triiodothyronine (T3) is generated from the deiodination of thyroxine (T4) by peripheral tissues. Using radioactive T3 as a tracer in serum uptake assays confirms the diagnosis of hyperthyroidism by measuring the increased uptake of T3.4 Increased serum levels of either hormone signify hyperfunction, while depressed levels indicate hypofunction of the thyroid. Simple determinations of T4 usually suffice to confirm suspected hypothyroidism; more demanding assays are needed in patients with altered thyroid-hormone binding proteins.

Steroid hormones of the hypothalamic-pituitary-gonadal axis can be detected in blood and other body fluids. The most commonly measured are estrogen and testosterone. In adult men, plasma testosterone levels reflect testicular function, while those in premenopausal women mainly reflect ovarian function. Abnormal concentrations are clinically relevant indicators of conditions in the respective endocrine organs [112, 113, 114, 115].

Chapter - 7

Plasma Enzymes

Major enzymatic groups in blood plasma include proteases, kinases, glycolytic and TCA-cycle enzymes, and leakage enzymes. Proteases are involved in extrinsic/intrinsic coagulation pathways, tissue remodeling, and inflammatory cascades. Kinases initiate signal transduction for metabolic control and vascular dynamics. Glycolytic and tricarboxylic acid cycle enzymes reflect systemic disturbance of glucose and energy metabolism. Leakage enzymes provide specific surrogates for tissue damage and disease involvement.

Only a minority of plasma enzymes are incorporated into diagnostic algorithms. Aligning detection with tissue origin, analyzing activity changes and ratios, and incorporating kinetic behavior into diagnostic models enhance the diagnostic potential.

Plasma enzymes participate in multiple groups of complex biochemical reactions; quantifying activity provides systemic information about patients and models. Major enzymatic groups in plasma can be classified according to their source (endogenous/ exogenous) and mode of action (native/catalytic). Native enzymes (proteins and glycoproteins) are present in plasma when active but do not catalyze physiological reactions; instead, they are structural constituents of cells, tissues, and organs and may leak into blood following damage. Active catalytic enzymes speed up biochemical reactions and mediate systemic homeostasis; thus, they can indicate metabolic disturbance and disease presence or extent. The three main groups are proteases, kinases, and leakage enzymes.

Cascades of plasma proteases underlie the extrinsic and intrinsic pathways of coagulation. Tissue-plasminogen activator participates in the localized fibrinolytic cascade along with plasminogen, which is converted to plasmin by tissue-plasminogen activator and urokinase, and the inhibitors plasminogen-activated inhibitor type 1 and type 2. Proteases also partake in complement-mediated inflammation, transforming growth factor-β activation, and matrix metalloproteinase-initiated tissue remodeling [116, 117, 118, 119].

Major enzymatic groups in plasma

Plasma enzymes can be classified according to their origin into those of the liver, pancreas, intestine, heart, muscle, bone, and other tissues and those produced by microorganisms. A more functional classification distinguishes between enzymes participating in coagulation and fibrinolysis, oxidative processes, and metabolism (e.g. catalase, aldo-keto reductases, arginase, or functional enzymes of the circulating cells). Other enzymes are dysregulated but do not contribute directly to normally operating pathways of metabolism.

Under physiological conditions, enzyme concentrations in blood are generally low and largely stable due to precise regulatory control mechanisms. Their leakage into plasma is therefore indicative of tissue damage, and enzymatic activity measurements form the basis of most diagnostic algorithms developed in clinical biochemistry. Such tests are most commonly performed using spectrophotometric techniques, but electrochemical, fluorimetic, and luminescent assay principles are also applied [120, 121, 122, 123].

Biological roles and catalytic mechanisms

Enzymes belonging to several classes participate in plasma and perform a multitude of different functions. Seven groups can be differentiated based on the general nature of the reaction catalyzed: oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases, and lacs. Except for lacs such as glutathione synthetase and cystinyl glycine synthetase, plasma enzymes are either excreted by cells in their

active form, obtained by leakage from damaged cells or formed as a result of the natural metabolism of some tissues. Plasma levels of most enzymes remain stable during health. Subsequently they act either locally or in distant tissues and their concentration in plasma is determined by the rate of delivery and the rate of removal and catabolism.

The catalytic machinery of enzymes is built up of amino acid residues that provide the appropriate complementary structures to the substrate, stabilizing its transition state, orienting it in the right direction and undergoing alternate protonation and deprotonation during the reaction. This modulates the intrinsic reactivity and governs the specificity of substrate-enzyme interactions. Because enzymes are shaped so as to bind substrates better than the products of the reaction catalyzed, any factor that speeds the approach of substrates, favors their binding or lowers the intrinsic reactivity of the substrate-enzyme complex will enhance catalytic efficiency. Plasma is a viable biochemical reaction medium. Both concentrations of substrates and enzyme activities are orders of magnitude lower than in cells and tissue compartments; the residence times of enzymes and substrates are long as far as kinetics is concerned; and rapid diffusion of small substrates, of greater concentration in plasma than in the tissues, tends to overcome the disadvantages inherent to this milieu [124, 125, 126, 127].

Enzyme leakage as a marker of tissue damage

Plasma contains enzymes of several major groups, classified according to their origins and biological functions. The normal concentration rattles of these enzymes are usually orders of magnitudes lower than those of their substrate concentrations. Leakage of any of these enzymes into plasma has a functional significance and helps diagnosis. When tissue undergoes necrosis, the plasma membrane is ruptured and the intracellular components of the tissue are released into the plasma. Many intracellular proteins in a tissue catalyze the same reaction, some of which may be enzymatically inactive or functionally superfluous. Others may regulate the biochemical reaction without

playing a direct catalytic role. A thorough understanding of the changes in the concentration of the plasma enzymes under various pathological conditions helps in developing a diagnostic strategy.

For any enzyme, the amount in the plasma is a net result of the rate of synthesis and the rate of clearance. Sudden changes in the concentration are difficult to interpret unless the two competing processes can be independently examined. Clinically enzymes present in the plasma are analysed using different assay methods. Although many assay techniques can be employed for the measurement of enzyme activity, the most common are based on either colourimetric or fluorometric principle. Each assay method has specific advantages and disadvantages. The choice of assay principle depends on the enzyme to be analysed, availability of reagents, standardisation and validation. Most important, the biochemical nature of the enzyme and its substrate need to be considered to avoid assay failures [128, 129, 130, 131].

Clinical measurement methods

Common laboratory tests measure the concentrations of plasma components, often using plasma-enzyme concentrations as diagnostic indicators. Enzyme concentrations can be assessed using various methods, including spectrophotometric, fluorescent, luminometric, chromatographic, and electrophoretic techniques.

Enzyme activity is usually measured at a defined temperature and pH. When a colorless substrate is transformed into a colored product, the enzyme-catalyzed reaction can be monitored by following the absorbance change at a suitable wavelength with respect to time. The activity of a dehydrogenase can be detected by coupling it with the oxidation of a coenzyme, resulting in a change in absorbance. If the reaction produces or consumes a gas, it can be detected with a suitable electrode. Other non-spectrophotometric approaches can also be employed to monitor the reaction.

Fluorogenic substrates can be used to determine various cleaving enzymes that are involved in blood clotting or degradation of the fibrin

mesh, as well as peptidases in the digestive tract. Covalent enzyme-cofactor conjugates can also be applied. In addition to the classical biochemical methods that have been widely used for decades, the use of mass spectrometry has gained widespread acceptance in the analysis of enzymatic activity, and targeted proteomic assays such as selected reaction monitoring have been used for quantification [132, 133, 134, 135].

Chapter - 8

Immunological Components

Changes in tissue integrity activate various immune responses in the body. Within blood plasma, innate immunity is mainly represented by the complement system and acute-phase proteins. The complement system is a series of over thirty plasma proteins that act in concert to opsonize pathogens, promote inflammatory reactions, and destroy cells. Cytokines secreted by activated immune cells induce the production of acute-phase proteins, which may enhance pathogen clearance, promote the resolution of inflammation, and protect the organism from septic shock. Antibody-mediated adaptive immune responses also occur in plasma. The establishment of immunological memory enables the detection and clearance of pathogens by specific antibodies produced in response to antigen exposure or vaccination.

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Antibodies and immunoglobulin classes

Plasma contains circulating antibodies (immunoglobulins) that constitute an essential part of the immune system. Antibodies are produced by B lymphocytes. During differentiation, B lymphocytes become immunoglobulin-secreting plasma cells. Plasma cells synthesize large amounts of antibodies that can specifically bind to the same (or similar) foreign antigen that stimulated the original B-lymphocyte clone. Antibody-binding to foreign antigens enhances phagocytosis and destruction of microorganisms and helps neutralize toxins produced by bacteria. The major classes of immunoglobulins in mammals differ in size and structure and are thought to have different and specialized roles in immunity. All classes of immunoglobulins form a similar basic molecular structure consisting of two identical heavy and two identical light polypeptide chains, except for the different types of heavy chain. Heavy chains are five classes of immunoglobulins: IgM, IgG, IgA, IgE, and IgD, classified on the basis of the type of heavy chain. The different classes of immunoglobulins contain variable and constant regions. The variable portions are at the tips of the antibody and are involved in antigen binding whereas the constant portions are conserved and differ for the classes. The other part of the antibody can be bound to the surface of mast cells and basophils. IgG antibodies account for approximately 70-75% of all antibodies in human serum. They participate in all immune reaction; are transferred from mother to foetus through the placenta and endow the infant with a passive immunity for the first year of life. IgM account for approximately 10% of the serum immunoglobulin. IgM antibodies are involved in the response to an early stage of infection by microorganisms and often appear first in serum. IgA, present in blood and body secretions such as saliva, tears and breast milk provides infectious disease protection by trapping antigens in body secretions. IgD, as a receptor has a role in activating B lymphocytes. IgE is present in extremely small quantities (0.002%). They participate in allergic responses [141, 142, 143, 144, 145].

Complement system components

The complement system constitutes a pivotal defense strategy of innate immunity, comprising distinct plasma proteins that participate in conspicuous interaction networks. Three activation pathways classical, alternative, and lectin culminate in the cleavage of C3 into C3b and C3a, whose respective properties and functions ignite potent effector mechanisms. C3b, in concert with additional components, enables opsonization and formation of the Membrane Attack Complex (MAC), whereas C3a, alongside other anaphylatoxins, amplifies inflammation, neutrophil recruitment, and chemotaxis.

An informative panel of complement genes is situated in a chromosomal region of the human genome rich in immune-inflected loci. Chimerical associations with certain diseases and vulnerabilities to infections serve as indirect yet reliable indicators of complement system function. In addition, complement levels and activities have been profusely exploited as diagnostic markers of various conditions. The biological complexity of the complement system is further reflected in its peptidic fragments, some of which are endowed with intrinsic antimicrobial activity, neuropeptidic functions, or roles in metabolic traces thus far neglected [146, 147, 148, 149].

Cytokines and inflammatory mediators

Released by blood cells act systemically through the circulation and contribute to the communication network that expands beyond a localized immune response. Cytokine cascades amplify processes associated with innate immunity, such as acute inflammation and wound healing, whereas signals of the adaptive response target T cells and B cells. Prostaglandins, bradykinin, and other mediators from damaged tissues and local innate immune cells can alter the activity of resident endothelial cells, pericytes, and smooth muscle cells, and promote systemic effects via the circulation by acting on the feedback or regulatory elements that activate or inhibit their production. Most cytokines have multiple effects and their functions partly overlap. Plasma concentration changes of several cytokines may occur during

the septic response, infection, tissue injury, malignancy, graft rejection, and chronic inflammatory diseases, especially rheumatoid arthritis and periodontitis. Multiplex methods allow many of these soluble mediators to be measured in small plasma samples with high sensitivity, medium specificity, and little time.

Cytokines are small secreted proteins released by cells that affect the behaviour of neighbouring cells. Initiation of inflammatory cytokine and chemokine production can be mediated by Toll-like receptors, spawn a complex cascade of different mediators, and act in an autocrine, paracrine, or endocrine manner. Acute- and chronic-phase proteins, other mediators of systemic inflammation, and growth factors released from non-immune cells may change the production pattern of cytokines. Cytokine profiles reflect and help to characterize disturbances in the immune system. The often-observed upregulation of pro-inflammatory cytokines such as interleukin-1β, interleukin-6 (IL-6), interleukin-10, interferon-γ, resistin, and tumour necrosis factor-α (TNF-α), alone or in combination with increased levels of C-reactive protein, represents a major deviation from homeostasis and has been implicated in the pathology of a variety of disorders [150, 151, 152, 153, 154].

Plasma-based immune diagnostics

Shifts in biological conditions result in the release of different antibodies for different invading organisms, and these antibodies can be used as a valuable diagnostic tool. Serology is the study of host immune responses to pathogens, and serological tests measure antibodies in serum or plasma. Detection of antigens can also indicate a recent infection. Other antigen-detecting studies include immunohistochemistry, which detects antigens in tissue samples, and reverse transcription quantitative PCR, which detects the RNA of viruses.

Serum or plasma can also be used in more complex immunoassays, such as the ELISA (enzyme-linked immunosorbent assay) and its multiple variations. ELISAs use immobilized antigens or antibodies in

a 96-well plate and subsequent antibody or antigen detection steps to quantify levels of the desired analyte. In a typical setup, the sample or calibration standard is incubated with the capture antibody, and then horseradish peroxidase-conjugated secondary antibody is added. The substrates are supplied for washing steps: tetramethylbenzidine is utilized for colorimetric detection, and 3,3′,5,5′-tetramethylbenzidine triggers chemiluminescence. These analytes are often multiplexed to conserve the precious biological sample and are spotted on membranes or beads.

Other groups of plasma components and several compounds that are released from cells involved in inflammation can also be used in immunoassays for investigational or diagnostic purposes. For example, C-reactive protein (CRP) and procalcitonin are secreted from the liver and the thyroid gland, respectively; they are positively associated with inflammation and sepsis. Levels of these molecules can be measured with ELISA. Enzyme activity profiling of serum or plasma is also valuable in diagnosing various conditions. Markers such as activity change in the presence of heart and liver damage, tissue disruption, hypoxic injury, and muscle metabolism are frequently monitored; these enzymes can be further exploited for blood doping surveillance. Other analytes that are frequently measured include proinflammatory and anti-inflammatory cytokines; increased levels can indicate high systemic inflammation [155, 156, 157, 158].

Chapter - 9

Coagulation and Fibrinolysis Chemistry

Complex pathways orchestrate hemostasis. When vascular endothelial cells are damaged, Tissue Factor (TF) is exposed and thromboplastin is released. Prothrombin is activated to thrombin, which cleaves fibrinogen to form fibrin strands. Thrombin amplifies the cascade through positive feedback loops and activates cofactors that accelerate the reactions.

Hemostasis is achieved through three physical events: vasoconstriction, platelet plug formation, and fibrin clot formation. Subsequent healing of the damaged endothelium renders fibrinogen, prothrombin, and tissue factor superfluous. Fibrinolysis, or clot breakdown, restores blood flow through the vessel when it is no longer required.

Clotting factors are primarily synthesized in the liver and remain inactive until catalyzed to their enzymatically active forms. Plasma factor concentrations can be markedly altered during pregnancy or disease. Deficiency of single factors leads to bleeding risk and specific screening tests are used to identify these deficiencies [159, 160, 118, 161].

Intrinsic and extrinsic pathways

The coagulation cascade can be divided into two main pathways: the intrinsic pathway and the extrinsic pathway. The intrinsic pathway is triggered by contact with negatively charged surfaces or damaged endothelial cells and is initiated by Factor XII (Hageman factor) and/or Factor XI (Plasma thromboplastin antecedent). These factors are activated to XIIa and XIa, respectively. This pathway consists of

several amplification steps in which, as seen in cascade systems, only a small initial signal leads to a considerable increase in formed product. The common endpoint of both pathways is the formation of Factor Xa.

The coagulation cascade is shown in detail in reaction 9.1. At the initial stages, coagulation proceeds through the intrinsic pathway. Factor IX (Plasma thromboplastin component), which is present in low concentration, is rapidly activated to IXa by Complex VIIIa (Factor VIIIa together with its cofactor Factor IX) formed from Factor VIII (Antihemophilic factor) by thrombin activation. Factor IXa in turn activates Factor X. Factor X (Stuart factor) can also be activated through the extrinsic pathway involving tissue factor, which is an integral membrane protein present in antigenically non-cross-reacting forms on the surface of extravascular tissues. Activation of Factor X by Factor VIIa (Factor VII in complex with tissue factor) takes place in the presence of calcium ions and the calcium-dependent membrane phospholipid complex that is formed during coagulation [162, 163, 164, 119].

Coagulation factor chemistry

The coagulation factors consist of a series of plasma proteins that, together, form a complex system for the repair of vascular damage. The extrinsic and intrinsic pathways activate a common pathway, leading to the conversion of the soluble plasma protein fibrinogen to insoluble fibrin, which polymerizes to form cross-linked fibrin clots. The synthesis of almost all factors occurs in the liver and the majority are synthesized as zymogen precursors. Proteolytic cleavage of the zymogens during the coagulation process activates the factors. Some factors also require an additional protein for activation. Thrombin is pivotal in coagulation, catalyzing multiple reactions along the cascade as well as activating factors V, VIII, and XIII.

The lytic system for breaking down fibrin clots (fibrinolysis) operates reversibly. Plasminogen is the inactive zymogen, which is converted to the active serine protease plasmin by plasminogen activators. Various enzymes and other plasma proteins regulate the processes. Abnormalities involving the coagulation and fibrinolytic

system can give rise to bleeding problems, thrombophilia, and a higher risk of thromboembolic disease [165, 166, 167, 168, 169, 165, 166, 167, 168, 169, 165, 166, 167, 168].

Fibrinolysis and clot breakdown

Plasma contains a number of specific proteolytic enzymes, collectively known as plasminogen activators, that initiate the breakdown of an undesirable clot and promote tissue remodeling. Plasminogen, a component of all plasma, is converted to the active proteolytic enzyme plasmin by two groups of plasminogen activators:

1) The tissue-type (T-PA) and

2) The urokinase-type (u-PA) plasminogen activator.

T-PA catalyzes the conversion of plasminogen to plasmin in blood clots and in the immediate platelet-rich layers of the clot, while u-PA is found in a number of tissues throughout the body. Once activated, plasmin catalyzes the digestion of fibrin and fibrinogen. It attacks other coagulation factors, including Factors V and VIII, and various other plasma proteins. Plasmin digests fibrin into small pieces called fibrin degradation products, which can appear in the urine.

Clots are also temporally modified by the activity of a plasminogen-activator inhibitor (PAI-1). PAI-1 is a single-chain glycoprotein found in plasma that inhibits T-PA and u-PA. It regulates fibrinolysis primarily by keeping plasmin activity in check. There are also specific plasminogen activator inhibitors capable of blocking the activity of u-PA, but these are present in the tissue itself, not in plasma. Another true plasmin inhibitor, α2-antiplasmin, is also found in plasma and appears to be the only factor capable of controlling plasmin activity in plasma, either making its presence or absence a marker of disease.

Disorders and diagnostic tests

The major electrolytes in blood plasma and their normal physiological concentrations are Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and HCO^{3-}. Imbalances in these ions are routinely detected and serve as indicators

for a variety of medical conditions. Nevertheless, a single abnormal value does not indicate a specific disease or condition; thus, results must always be interpreted within the overall clinical context and in conjunction with results from other relevant tests.

By far, the most abundant extracellular cation is Na^+. Approximately 2500 mEq of Na+ is located primarily in the interstitial fluid and plasma, where it constitutes more than 90% of the cations. The physiological effect of Na+ is to control osmotic equilibrium and maintain the normal distribution of fluid between the extracellular and intracellular compartments. The Na^+ concentration is regulated chiefly by the renal system. Alterations are termed hypernatremia and hyponatremia, and both conditions can lead to serious metabolic consequences. Hypernatremia usually results from excessive water loss, whereas hyponatremia is commonly due to excess water and reduced salt intake or excretion.

The second most abundant cation in plasma is K+, constituting about 40% of the total cation concentration. K+ participates in the regulation of resting membrane potentials and hence in excitability of nerve, skeletal muscle, and cardiac muscle. Tight control of plasma K+ concentration is essential to normal function. Hyperkalemia and hypokalemia may produce potentially lethal cardiac arrhythmias. K+ homeostasis is primarily regulated by the kidneys and influenced by aldosterone and plasma [H+].

Chapter - 10

Plasma Electrolytes and Mineral Balance

Electrolytes are fundamental plasma constituents that ensure physiological homeostasis. The major electrolytes in blood plasma include sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), chloride ($Cl^–$), magnesium (Mg^{2+}), and bicarbonate ($HCO^{3–}$). Sodium is present at 135-145 mmol/L and critical in maintaining osmolarity and water homeostasis. Potassium serves as the main intracellular cation and is central to resting membrane potential and cardiac excitability, with a normal serum concentration of 3.5-5.0 mmol/L. Calcium is crucial for blood coagulation cascade activation and muscle contraction, existing in protein-bound, ionized, and complexed forms (normal range: 2.12-2.60 mmol/L). Chloride is the major anion in extracellular fluid, contributing to maintaining osmotic pressure (reference range: 96-108 mmol/L). Physiological Mg^{2+} (0.65-1.05 mmol/L) is essential for enzymatic catalysis and neuromuscular transmission. Bicarbonate is a key buffer in maintaining acid-base balance.

Homeostatic regulation of plasma electrolytes occurs at multiple levels: renal control adjusts tubular reabsorption and excretion, endocrine effects mediate sodium and calcium balance, and cellular transport mechanisms modulate extracellular concentrations. The bicarbonate buffer system plays an important role in acid-base equilibrium, with arterial blood $HCO^{3–}$ levels reflecting metabolic disturbances. Correct identification of altered serum electrolyte concentrations assists in establishing diagnoses, guiding treatment, and monitoring disease progression.

Major electrolytes and their roles

The major electrolytes in blood plasma are sodium, potassium, calcium, magnesium, chloride and bicarbonate. Sodium is the principal cation and the largest contributor to plasma osmolarity; it is involved in the generation of action potentials and maintains fluid balance. Potassium is the most abundant cation inside cells, where it is primarily involved in establishing the resting membrane potential. Calcium is essential for the release of neurotransmitters and muscle contraction; it regulates coagulation and serves as a second messenger. Magnesium is a cofactor for many enzymes, including those in the glycolytic pathway, citric acid cycle and nucleotide biosynthesis. Chloride is the major anion inside and outside cells; it participates in the formation of gastric acid and is transported in exchange for bicarbonate during carbon dioxide transport. Physiological bicarbonate levels and the associated buffering capacity are vital for acid-base homeostasis.

These electrolytes are kept in a narrow range by various regulatory mechanisms, including the actions of the kidney, endocrine glands and cells. The kidneys eliminate excess ions and reabsorb deficient ones, while hormones like aldosterone act on the kidney; parathyroid hormone, calcitonin and vitamin D modulate calcium; and insulin influences potassium. Interactions between bicarbonate and CO_2/CO_3 2−atmospheric acid-base equilibrium underpin acid-base balance. Electrolyte imbalances can affect cellular homeostasis, organ system function and human health. The concentration of any electrolyte in plasma can serve as a clinical marker, and deviations from the normal ranges in these electrolytes are among the most urgent considerations in hospitalized patients [170, 171, 172, 173, 174].

Homeostatic regulation mechanisms

Utilization of cellular and environmental pH sensors maintains acid-base homeostasis, and imbalances can arise from hypocapnia, hypercapnia, renal diseases, profuse sweating, vomiting, diarrhea, and titration of metabolic acids or bases. Congestion in metabolic pathways produces excess acids or alkaline components that alter overall

balance. Inadequate kidney filtering of urea, uric acid, phosphates, sulfates - or acids resulting from fat or carbohydrate breakdown - causes acidosis, while removal of excessive base-forming alkalosis-contributing ions results in alkalosis.

Oxide ions shift the equilibrium of HCO^{3-} formation and reaction-diffusion process for hydration-dehydration of CO_2, so that carbon dioxide diffusing from tissues to blood, plasma, and luminal epithelium of pulmonary alveoli explodes when released. Deoxygenation of hemoglobin accelerates the rate of carbon dioxide absorption. Disturbances in acid-base buffer systems can arise from renal diseases, respiratory diseases, structural disorders of blood vessels, or infection-induced increase in temperature or consumption of alkaloid or acid constituents, but buffers minimize and sustain changes [175, 176, 177, 171, 178].

Acid-base balance interactions

Acid-base interactions of plasma with other body fluids should be considered, particularly with regard to bicarbonate buffering and pH maintenance. The cycling of carbonic acid in addition to the Henderson-Hasselbalch equation reflects the essential physics of blood pH homeostasis. Bicarbonate serves as the major blood buffer, and true metabolic acidosis/alkalosis therefore involves alterations in $SCMCO_2$, whether from a respiratory cause/compensation or metabolic cause/compensation. Other plasma buffering systems also influence acid-base balance, but are usually of secondary importance compared with respiratory and kidney-dependent alterations.

Blood CO_2 and water osmolarities may move in opposite directions under certain conditions owing to hypercapnia, which induces respiratory acidosis and causes CO_2 diffusion into cells. The influence on acid-base balance is multifaceted, with greater importance during respiratory diseases and hypercapnic ventilatory failure. A secondary role in physiological states may be described as accommodation or adaptation to changes in HCO^{3-} concentration or pH, and removing a key anion may lead to consequences such as differential H+

distribution. An understanding of these processes can assist clinicians when evaluating respiratory disorders and the causes of gammapathies. Impairment of the relationship between pH and PCO_2 during metabolic acidosis and alkalosis may also assist in the diagnosis of differential pathophysiology [174, 179, 180, 181, 182].

Electrolyte disorders and clinical markers

The major plasma electrolytes sodium, potassium, calcium, magnesium, chloride, and bicarbonate fulfill crucial physiological roles, including establishing osmotic pressure, driving the electroneutrality of body fluids, and regulating acid−base balance. These functions are tightly controlled via complex homeostatic mechanisms involving the kidneys, endocrine system, and cellular actions. Disturbances in the concentration of key electrolytes may compromise basic physiological processes and subsequently lead to organ dysfunction. Plasma concentrations of the minerals copper, iron, and phosphate also require careful regulation, although disorders of these elements are less common.

Analysis of plasma levels remains an essential tool in the diagnosis and clinical management of many electrolyte disturbances. Hyponatremia and hypernatremia are recognized markers for body fluid disorders, hyperkalemia is a frequent warning of renal failure, hypercalcemia may result from malignancy, and dysregulation of magnesium metabolism is often associated with systemic inflammation [183, 184, 185, 186].

Chapter - 11

Plasma as a Transport Medium

Dissolved in plasma, gases such as carbon dioxide and dissolved nitrogen exist in a free state. The oxygen molecule is hydrophobic and needs a transporting protein, myoglobin or hemoglobin, to get in and out of the respiratory system and reach the tissues. Myoglobin serves this purpose at the level of the muscle and carries the most elevated concentration of oxygen in the body. Hemoglobin, composed of four αβ dimers, forms a reversible complex with the oxygen molecule. As such, hemoglobin can bind to four molecules of oxygen and can even release it again in a reversible and controlled fashion depending on pH, temperature, carbon dioxide, and 2,3-bisphosphoglycerate concentration. Although the binding constant of the hydrogen ion is lower than that of the oxygen molecule, the great concentration of the latter, sometimes five times higher than that of the hydrogen ion per unit of volume, allows the saturation of hemoglobin with oxygen. On entering a highly metabolically active tissue, hemoglobin uniforms pH because it binds hydrogen ions more tightly than oxygen. This favoring gradient of hydrogen ions from the tissues toward the blood that increases the partial pressure of CO_2 is important for the excretion of the respiratory acid product of the oxidation of food.

The plasma milieu is crucial for the delivery and the removal of nutrients and waste products of the metabolism of the cells. In particular, glucose and lactate enter the glycolytic pathway, while the lactate produced at the level of glycolysis passes to the liver and is converted into glucose in a process called gluconeogenesis. Triglycerides produced in the liver, adipose tissue, and intestine are

transported by lipoproteins in the bloodstream, and their hydrolysis catalyzed by lipoprotein lipase releases free fatty acids, which are taken up by all the cells and enter directly in the mitochondrial β-oxidation, the mains supplies of energy in the muscle and in the liver. Urea and creatinine are waste products of the metabolism eliminated by the kidney. They are also permeable and pass freely through the glomerular membrane and, therefore, can act as a marker of kidney function. Drugs are also plasma constituents: when entering the systemic circulation, they are distributed to all tissues and organs, and their action is the result of a complex equilibrium with the extracellular fluid. Plasma proteins can help or disturb drug action: the dose-response curve can be displaced to the right or to the left depending on the binomial relationship established by the drug with plasma proteins [187, 188, 189, 190].

Gas transport and dissolved components

Gas transport in blood plasma occurs mainly via hemoglobin-bound oxygen and bicarbonate-dissolved carbon dioxide, with minor contributions from free O_2, CO_2, and N_2. Following lung and tissue perfusion, O_2 and CO_2 gradients establish diffusion mechanisms for the gaseous exchange. Anaerobic glycolysis in the cytoplasm generates lactic acid, which dissociates into lactate and H+ and reduces the plasma pH. These kinetic shifts stimulate breathing increase facilitated mainly by pre-Bötzinger neurons in the medulla oblongata, whereby pH is restored to physiological ranges.

Apart from gases, plasma principally carries metabolic substrates, electrolytes, small-molecule metabolites, and waste products between tissues. Non-polar solutes present in low concentrations associate with plasma-transferring proteins through specific and non-specific interactions. Such binding influences intracorporeal distribution and metabolic transformation rates and significantly alters the apparent half-life of drugs with high protein affinities. In addition, haptoglobin, hemoglobin, transferrin, and lactate dehydrogenase quantitatively express protein concentration variations caused by certain diseases [57, 191, 192, 193, 57, 191, 192, 193].

Nutrient and waste transport

Plasma plays a vital role in transporting and delivering nutrients to tissues and removing metabolic waste products and other by-products of cellular metabolism from tissues. Carbohydrates, amino acids, ions, and small molecules, as well as proteins, lipids, and lipoprotein particles, are soluble in the hypertonic liquid environment of the bloodstream and can, therefore, diffuse across the walls of capillaries into tissue fluid. The diffusion process is facilitated by differences in concentration across the capillary wall.

The major soluble metabolic waste products released into the plasma by tissue cells include ammonia, urea, creatinine, uric acid, carbon dioxide, and bile pigments. Ammonia is produced mainly during amino acid catabolism. Urea and uric acid are excretory products formed in the liver and eliminated in the urine. The excretory products urea, creatinine, uric acid, and ammonia are toxic in excessive concentrations, and their detection in plasma at increased concentrations is an important sign of renal failure.

Drug binding and pharmacokinetics

The plasma milieu plays a key role in the transport of therapeutic drugs throughout the body and in their respective pharmacokinetics. Many drugs naturally occur in plasma in free or unbound form, but the majority are bound to plasma proteins such as albumin and α1-acid-glycoprotein. Free drug concentrations dictate the biological activities typically associated with drugs, thus the proportion of the drug that is protein-bound is a major determinant of drug activity, especially for anti-infective and anticancer drugs. Drugs that are highly protein-bound (typically >90%) have a slow rate of distribution and a low volume of distribution, whereas drugs with lower protein binding may have large volumes of distribution and more rapid rates of distribution.

During drug development, understanding the extent and driving forces of plasma protein binding is essential for assessing therapeutic window for efficacy and toxicity, and in determining initial dosing

regimens. Concentration-dependent changes in drug clearance and distribution volume at therapeutic concentrations may necessitate dosing alterations or formulation changes (e.g., intravenous versus oral route) to effectively treat patients. Special attention must also be given to the analysis of drug-drug interactions that alter free concentrations of highly protein-bound drugs, as such alterations may lead to serious and possibly life-threatening adverse effects. Systematic reviews of protein binding at the extremes of age, hepatic and renal failure, and critically ill patients help to understand these complications in a clinical context. For many drugs, the role of protein binding in influencing unbound concentrations is pivotal in determining pharmacological effect and toxicology [40, 194, 41, 195, 40, 194, 41, 195].

Specialized carrier proteins

A small set of proteins binds nutrients and maintains their low plasma concentrations within the range needed for cellular delivery. Transferrin, which contains a high-affinity iron(III) binding site, transports iron; its concentration adapts to the iron status of the organism. Unconjugated bilirubin is carried by a different binding site on albumin. Specific binding sites are also present for fatty acids, thyroid hormones, retinol, and certain anions (e.g., ferric ammonium citrate). These specialized carriers facilitate the movement of their respective ligands across physiological membranes. Lipoproteins can be envisioned as shell structures that enable the transport of lipids; they deliver triglycerides and cholesterol to tissues and facilitate the excretion of excess cholesterol from the body.

Chapter - 12

Interactions Between Plasma Components

The plasma milieu provides an active environment for the formation of protein complexes, the assembly of lipid-protein particles, and the impact of enzymes on substrates. Reevaluation of complex formation offers a more holistic view of plasma biology.

Protein-protein interactions are common in plasma. Transferrin, ferritin, and haemopexin bind iron and heme, respectively, while a range of proteinase inhibitors complex with serine proteases and cysteine proteases. Other examples include the complement system, tissue factor-stage 3 complex, and antibody-antigen combinations.

Lipid-protein complexes characterize plasma. A phospholipid monolayer-surrounded triglyceride-rich core forms the structure of chylomicra, Very-Low-Density Lipoproteins (VLDLs), and Intermediate-Density Lipoproteins (IDLs). Apolipoproteins influence metabolism and fate, enabling chylomicra and VLDLs to bind to the cellular lipoprotein lipase, while the ApoE moiety of IDLs interacts with cell surface receptors. Heterogeneous High-Density Lipoprotein (HDL) particles carry apolipoprotein A along with sphingomyeline and phosphatidylcholine. The spherical lipid-protein assembly is implicated in tissue cholesterol transport.

Enzyme-substrate interactions in plasma can be limited by the inherent low concentration of substrates, resulting in minimal turnover in the sample. Catalytic efficiency must therefore take into account not only velocity, but the relative concentration of substrate within the plasma volume. Hemoglobin thus possesses low kcat values for the

reduction of plasma nitrites to nitric oxide, which in physiological conditions would appear to be too low to promote such a reaction, until the inherent concentration of hemoglobin is considered.

The plasma milieu represents a complex network of multiple components, whose integrative action contributes to the overall functionality of the plasma. The concerted metabolic impact of both the plasma proteome and metabolome upon maintaining homeostasis serves as a technically expedient example of such interactions. Despite the intricacy of these interrelations, the principal substrate categories of the biological pathways are relatively well defined [196, 197, 198, 199].

Protein-protein interactions

(PPIs) profoundly influence plasma composition and functionality by modulating stability, dilation, and biological activities. Binding partners include hormones, cytokines, enzymes, and immunoglobulins. Formation of hormone-receptor complexes transduces signals from cell communication. Cytokines secreted by activated immune cells act on other immune cell types, and produce systemic effects under pathological conditions such as sepsis. Other mediators regulate coagulation and fibrinolysis, repair tissue injury, and facilitate healing. Binding hormones, such as insulin-like growth factor, leptin, and partially erythropoietin, enhance biological potential. The bloodstream serves as a reservoir for cytokines and triggers systemic responses during severe infection and trauma. Cytokines have been proposed as potential clinical markers for predicting severe infectious diseases.

Immunoglobulins engage in pathogen recognition and neutralization through specific PPI. Antigen-antibody complexes, immune complexes, complement-antibody interactions, and formation of immune nodes, such as granulomas, complete the immune response. Plasma also harbors time-limited inflammatory signals. The complement cascade generates pro-inflammatory mediators that orchestrate an acute-phase response to tissue insults. In addition, pro-inflammatory cytokines stimulate acute-phase protein synthesis in hepatocytes, while allowing sensitive, unbound plasma cytokine

detection and offering access to information on immune status, without relying on physical access to sampled tissues. Low-abundance signaling molecules trigger distinct defensive decisions across the organism, a feature exploited by the signalling network as a whole through the engagement of pathogen-associated molecular patterns [52, 54, 200, 201].

Lipid-protein complexes

are key structural and functional components of blood plasma. Plasma lipid classes include free fatty acids, triglycerides, cholesterol, sphingomyelins, and phospholipids. Their concentrations are influenced by tissue metabolism and systemic interactions, particularly with lipoprotein particles. Lipoproteins are spherical macromolecular lipid-protein complexes with an inner lipophilic lipid core and a surface shell made of amphipathic lipids and apolipoproteins. Their major classes chylomicrons, very-low-density lipoproteins, low-density lipoproteins, and high-density lipoproteins differ in size, composition, metabolic origin, and tissue distribution.

Cholesterol is a fundamental component of eukaryotic plasma membranes, conferring fluidity and structural stability. Apolipoproteins embedded in the lipoprotein surface reside in the peripheral layer and prevent particle coalescence and rapid hepatic clearance. Notably, advanced atherosclerosis and Alzheimer's disease are associated with dysregulation of lipoprotein cholesterol metabolism and homeostasis.

Specific plasma components modulate transport of drugs and other xenobiotics with known pharmacological effects. Lipoproteins can affect the pharmacokinetics and pharmacological effects of lipophilic drugs, and either facilitate or inhibit the distribution and action of other therapeutic drugs by altering their affinity for binding to plasma proteins. For example, the presence of certain lipoprotein subfractions can enhance the efficacy of taxanes in breast cancer patients [202, 203, 204, 205].

Enzyme-substrate interactions

Plasma enzymes frequently facilitate their reactions in concert with one or more protein partners, thereby forming multi-component complexes. These are common in dimeric enzymes, such as ornithine decarboxylase, and in enzymes that are fully functional only when bound together in a multimer (tryptophan pyrrolase). Such interactions may also enhance catalytic efficiency. For example, the semi-quantitative analysis of the relative diffusion-controlled kinetic efficiencies of tryptic cleavage of 82 natural substrates provides convincing evidence that binding with the E1 component of the pyruvate dehydrogenase multi-enzyme complex, which substantially augments the Kcat of the reaction, creates a reaction-gating effect, as these substrates present steric hindrance to the E2 component of the complex.

The interaction of classical enzymes with their substrates in the plasma milieu is interesting from the perspective of catalytic efficiency. The total concentration of a substrate in physiological fluid is, of course, the sum of the concentrations of the free substrate and the substrate-enzyme complex. The compartmentalisation of plasma is not tight. As a consequence, the individual components of a bimolecular reaction are not confined by surrounding barriers from diffusing past each other. As a result, the accessibility of substrate molecules to their cognate enzymes is likely not an all-or-nothing situation and, for classical enzymes acting on classical substrates, plasma is probably highly permissive with respect to the substrate pools. Consequently, when studying the distribution of classical substrates in the plasma milieu, the reactivity of a substrate molecule with its cognate enzyme may not be the sole factor controlling its total concentration. Therefore, it is reasonable to consider the interactions of the classical enzymes with their substrates in the plasma milieu mainly in terms of the second-order rate constant for association with the disorder [206, 207, 208, 209, 210].

Multi-component biochemical networks

A comprehensive understanding of blood plasma depends on

characterizing and connecting its numerous constituent components. Although described individually in the preceding sections, plasma proteins, lipids, small-molecule metabolites, hormones, enzymes, antibodies, and complement factors do not occur in isolation; rather, they participate in a network of interactions central to human physiology and pathology.

Protein-protein interactions account for the majority of these connections, affecting transport, storage, enzymatic, and regulatory functions. Many soluble plasma proteins possess specific binding partners that regulate their function or modulate physiological responses. For example, the concentration of free iron an essential element for various biochemical processes but also a potent catalyst of reactive oxygen species generation is controlled by the iron-binding plasma protein transferrin. Likewise, the binding of free fatty acids to albumin is critical for regulating their transport and preventing lipotoxicity. Plasma enzymes also participate in binding interactions; among the serine proteases, for instance, the specificity of plasmin is determined in part by the structure of its binding site. Coagulation cascades serve as another example, with several common co-factors required for multiple intrinsic and extrinsic pathways. The competence of the target enzyme governs the catalytic efficiency of the interaction: for low-affinity substrates, e.g., Chymotrypsin-Fibrin, the turnover rate is limited by substrate availability, whereas for high-affinity substrates, e.g., Antithrombin II-Thrombin, the reaction rate is determined by catalytic power.

Lipids and proteins also form complex assemblies in plasma, including lipoproteins, plasma membranes, and lipid rafts. Apart from their core-shell structure, with a lipid core and phospholipid-cholesterol surface bilayer, lipoprotein particles can be viewed as scaffolds displaying apolipoproteins that mediate important biological functions. For example, Apo-C1 and Apo-C2 direct lipoprotein metabolism by interacting with the endothelial-bound lipoprotein lipase complex. Lysophospholipids produced through lipoprotein metabolism can activate peroxisome proliferator-activated receptor γ

(PPARγ) and endothelial lipase, which functions as a phospholipase. In this way, lipoproteins serve not only as carriers of hydrophobic lipids but also as platforms that facilitate lipid metabolism and metabolism-related signaling. These insights highlight plasma as a unique environment for multidimensional biological interactions, with a single particle capable of participating in multiple pathways (lipid metabolism, immune response, and anaphylaxis).

Enzyme activity in plasma also reflects integrative influence. In general, plasma constitutes a physiological environment for the vast majority of common reactions: temperature, pH, allosteric regulation, and concentrations of cofactors are appropriate for in vivo catalysis. However, local and non-specific effects can alter enzyme performance. Assignments of relative substrate affinities are therefore approximations of reactivity rather than exhaustive predictions. Substrates not added at physiological concentrations are generally not limiting; substrates, co-substrates, and products added to plasma at lower concentrations than Kms normally drive reaction rates close to maximum. Furthermore, enzymes occurring in sufficiently high concentrations are often able to convert sure substrates rapidly [196, 211, 196, 211, 212, 213].

Chapter - 13

Techniques for Plasma Collection and Analysis

This overview of blood plasma presents an interdisciplinary integration of biology and chemistry that captures the many functions of plasma, the identity of its main constituents and metabolites, and their relation to disease processes. The concept of blood plasma has multiple facets: its chemistry is governed by laws and properties of chemical systems; its biology reflects the organism's state and regulation of homeostasis; and this perspective of blood plasma as a biochemical, chemical, and biological integrated system is widely used in disease diagnosis.

Plasma is obtained from whole blood by centrifugation in the presence of an anticoagulant. Major chemical studies focus on the malleable properties of blood plasma as a "supporting reactor mixture," that is, a medium containing all components of a complex biochemical reaction system at reasonable concentrations and constant composition, appropriate for a variety of biochemical reactions characterizing the different forms of life. Specialized spectroscopic and chromatographic techniques enable the study of low-abundance plasma components. The combined concentration/abundance gradient, an experimental concentration/abundance gradient helped to define and choose the proper methodology for the analytical study at those extreme concentrations. The analysis of diagnostic methods is focused on plasma, with attention given to commonly measured analytes, their physiological significances, and their alterations in different types of disease or environmental stress situations.

Plasma separation and storage

Blood plasma is an acellular fluid, generally separated from whole

blood using centrifugation. Blood is collected with anticoagulants to avoid coagulation. The choice of anticoagulant is generally the initial determinant for subsequent analysis; selected anticoagulants should not interfere with subsequent analyses. Blood cells and cell debris are rapidly pelleted by centrifugation (400-4,000 × g, 5-30 min), leaving plasma, which is then stored as above. Long-term plasma storage can be at -20 to -70 °C. Cryoprotectants and long-term storage at -80 °C or lower are not essential but can improve recovery and stability.

Plasma proteins are traditionally studied with spectrophotometric techniques (UV absorbance), electrophoresis, and immunoreactions. A contemporary approach combines proteomics (High-Performance Liquid Chromatography; Mass Spectrometry) with metabolomics, lipidomics, and transcriptomics (analysis of plasma-derived RNA, DNA, and protein basemarker detection at either mRNA or protein level).

Spectroscopic techniques

A variety of spectroscopic techniques have been employed for studying blood plasma and its constituent components. Spectroscopy encompasses techniques that measure the interaction of electromagnetic radiation with matter. Spectrometric methods are classified according to the radiation source and the manner in which matter interacts with it.

Optical spectroscopy exploits discrete transitions between quantized states of atoms and molecules. Ultraviolet (UV)-visible absorbance and fluorescence spectroscopy utilize excitations of σ, π, n, and d electronic orbitals, while Infrared (IR) absorbance spectroscopy employs vibrational transitions associated with covalent bond scissions. Raman scattering is based on inelastic scattering involving molecular vibrations. Nuclear Magnetic Resonance (NMR) spectroscopy utilizes magnetic dipole interactions and is a unique optical technique that probes the atomic magnetic moment of nuclei in a strong external magnetic field. Atomic Absorption Spectroscopy (AAS) exploits the absorption of UV/visible radiation associated with

transitions of metal ions from a lower to a higher electronic-energy state. Atomic Emission Spectroscopy (AES), on the other hand, measures the radiation emitted by excited species returning to the ground state. Mass spectrometric detection relies on determination of the mass-to-charge ratio of ions formed from targeted gaseous molecules [214, 215, 216].

Chromatographic methods

Chemical analysis of blood plasma employs chromatographic techniques for detailed qualitative and quantitative information. High-performance liquid chromatography (HPLC) is often combined with UV-Vis detection and is widely used for organic acids and carbohydrates. In HPLC, a mobile phase moves through a stationary phase fixed in a column, with differential interaction toward both phases by solute compounds leading to different retention times and separation. Liquid chromatography coupled with mass spectrometry (LC-MS) combines the separation capacity of HPLC with accurate mass determination capabilities of mass spectrometry, enabling the identification and quantification of metabolites of broad classes in a single analytical run.

Targeted metabolomic studies aim to measure a defined set of metabolites relevant to specific biochemical pathways, typically employing stable isotope-labelled internal standards. Metabolite concentrations are compared to calibrated responses for known standards. Non-targeted metabolomics embraces the intricate chemical composition of plasma with the goal of identifying as many metabolites as possible, often using highly sensitive and selective precursor and product ion monitoring or collision-induced dissociation. While non-targeted analyses do not require prior knowledge of metabolic concentrations, data interpretation is more difficult. More challenging analyses of lipid metabolites and lipid-associated metabolites take advantage of the lower complexity of plasma-lipid-related compositions and the greater independence of lipid metabolism from other pathways.

Proteins in plasma can be characterized by zone or specific activity-based assay electrophoresis. Zone electrophoresis utilizes the different charge-to-mass ratios of proteins to separate them, followed by densitometric area quantitation. Zone gel electrophoresis efficiently separates native proteins on the basis of mass, size, or both, while allowing detailed visualization of protein isoforms. Capillary-return-thermostatic slot immunoblotting exploits the higher resolution of capillary electrophoresis to enable densitometric or semiquantitative analysis of multiple proteins in a small amount of plasma. Antibody-based assays use the highly specific binding of antibodies for plasma proteins, linking immunogenicity to protein concentration and endogenous-clearing capacity [132, 217, 218, 219, 132, 217, 218, 219].

Electrophoretic and immunological assays

Electrophoresis, a versatile laboratory method, employs an external electric field to separate charged macromolecules in an electrolytic medium. Protein molecules are fractionated in a gel matrix with agarose or polyacrylamide, and nucleic acids or small spherical proteins are separated in a buffer for the hydrolysis reaction. Electrophoresis achieves high resolution due to the optimally chosen combination of supporting medium, electrophoretic environment, chemical properties of workspace and investigated substances. Nucleic acids, proteins and their mixtures are analysed with standard methods and procedures. Immunochemical techniques based on electrophoresis enable the detection of antigens and antibodies in various test objects.

Western blot detects specific antigens in protein extracts separated by electrophoresis. Capillary Electrophoresis (CE) is a separation method in which a buffer-filled capillary is placed in an electric field and negatively and positively charged components migrate towards the anode and cathode, respectively. CE has an order of magnitude higher separating power compared with gel electrophoresis, and it also requires far smaller sample and reagent volumes. Enzyme-Linked Immunosorbent Assay (ELISA) is widely used to detect specific antigens or antibodies in a sample. Various types of ELISA are

available. In direct ELISA, a layer of immobilized antibodies detects coated proteins. In non-competitive ELISA, unlabeled proteins in the sample are captured by immobilized antibodies, followed by the addition of enzyme-labeled antibodies. In competitive ELISA, sample analytes compete for binding to the immobilized receptor.

Chapter - 14

Cellular Communication via Plasma Components

Hormonal signaling pathways convey information between endocrine cells and target cells; multiple communicatory systems use plasma constituents to coordinate activities throughout the organism. Production of peptide and protein hormones occurs in specialized glands (e.g., the pituitary and pancreas) or in responding cells, and the chemicals travel via the blood to receptors in target cells. Control of release is primarily by negative feedback. For example, rising blood glucose levels stimulate insulin secretion by β-cells in the pancreas, enhancing cellular uptake, storage of glucose as glycogen, and conversion of glucose to fatty acids, thus returning plasma glucose levels toward homeostasis.

Cytokines orchestrate immune responses and coordinate the establishment, maintenance, and dissolution of inflammatory processes. Activation or stimulation of cells in the immune system induces production of pro-inflammatory mediators, with the acceptor cells being non-responding cells nearby or from a distance. Many of the cytokines enter the circulation, and some circulate below the limits of detection until they are required. The signals maintain the complex balance between immune modeling, immune activation, and immune resolution.

Extracellular vesicles are lipid-bound membranes that facilitate intercellular dialogue in a variety of systems and contexts; exosomes are a specific subset of the extracellular vesicles. Subpopulations of extracellular vesicles and exosomes can be distinguished based on their cargo. Elucidation and dissection of the topology of production,

transport, release, and uptake of extracellular vesicles facilitates understanding of their roles in normal physiology and policy-making and breakdown in a wide spectrum of diseases.

The orchestration of biological functions in different tissues and organs of living organisms is governed primarily by the endocrine system, which employs hormones as signaling molecules. Hormonal information flows from endocrine cells to target organs via the circulation where it exerts its biological action. In addition to hormones of classical endocrine glands, many cells and tissues secrete biologically active molecules that enter the circulation; these autocrine and paracrine signaling molecules reach distal target cells by being transported in the blood. Such signals include immunological mediators such as cytokines, chemokines, and growth factors [220, 101, 221].

Hormonal signaling pathways

Connect plasma hormones to target cells and elicit specific responses. Hormones affect virtually all physiological processes, including growth, metabolism, sexual function, reproduction, and sleep. Although they are secreted by discrete glands and tissues, interactions occur in the entire organism. Hormones can exert their effects even at low concentrations, but their roles in intracellular signaling are complex and involve multiple receptors and pathways, with several loops constituting feedback mechanisms that modulate endocrine regulation during homeostasis.

Essentially all endocrine hormones affect the blood plasma, and all outcome hormones act on the blood. Hormonal imbalance results in numerous diseases and extended pathways that trace hormonal production and degradation and clarify the pathway of their action in target organs. Circuit connections can therefore cover many systems such as the Central-sympathetic-Adrenal Connection (C-S-A Connection), the Gastro-Intestinal Center Connection (GIC C), the H-P-G Connection, and theGlucagon-like Peptide-1/(Oxy)ntomodulin Connection [222, 223, 224].

Cytokine-mediated immune communication

Cytokines are a large family of small soluble proteins that regulate immune cell differentiation, proliferation, and function. They are released by many cell types and act on neighboring or distant targets by engaging specific receptors, usually on plasma membranes. Cytokine binding activates intracellular signaling cascades through transcription factors that regulate effector gene expression. Cytokines mediate innate immunity and activate the adaptive immune response. Together with other signals, cytokine concentrations at the site of infection determine the T cell response and shape the host's development of protective immunity. Plasma is the primary route of systemic signaling, and cytokines released from tissues into the circulation can also elicit remote effects on many target organs.

The innate immune system, which provides the first line of defense against pathogen invasion, produces cytokines early during infection. Moreover, sensing persistent antigen triggers sustained secretion of pro-inflammatory cytokines, inducing tissue immune dysregulation and the development of non-communicable diseases. The acute-phase response to infection is governed by cytokines derived from innate immune cells such as macrophages, monocytes, and dendritic cells. In addition to local effects, systemic release of cytokines orchestrates the acute-phase response, stimulating effector leukocyte recruitment to secondary lymphoid tissues, tissues affected by infection, and areas of resolution [225, 226, 227].

Extracellular vesicles and exosomes

Cells communicate with each other via different kinds of chemical signals. Organ systems regulate and coordinate physiological responses through the blood circulation, a liquid medium in which signaling molecules (including hormones, cytokines, and other kinds of ligands) travel from the release site to rapidly reach and reach their target tissues or cells. In addition to signaling molecules, plasma also contains Extracellular Vehicles (EVs), exosomes, and membrane-derived nano- and micro-particulated vesicles that have an important

role in near-cell communication, contributing to the exchange of physiologically relevant materials, including neurotransmitters and other factors that control the activity of surrounding neighbor cells. EVs serve as systemic conveyors of pro-inflammatory, pro-fibrotic, anti-FH-, or tumor-promoting signals, and some of their cargos may serve as biomarkers for disease severity and prognosis.

Different cells in the organism "listen" each other according to the concentration of the local signals. For example, the concentration of the Lipopolysaccharide (LPS)-binding protein in the blood vesicles secreted by monocytes/macrophages, the local LPS concentration and the plasma-transported levels of cytokines (mainly pro- and anti-inflammatory cytokines) modulate the signals local macrophages receive from LPS. EVs encapsulate several classes of molecules, including proteins, lipids, and nucleic acids, and play a critical role in innate immune communication. During the inductive phases of an acute inflammatory response, these components of the innate immune system dynamically communicate with the peripheral immune effector cells, particularly myeloid lineage cells, to program the behavior of the myeloid cells engaged in the local inflammatory response [228, 229, 230].

Systemic regulation and feedback loops

Hormonal homeostasis regulates vital parameters in the body, such as growth, metabolism, stress responses, and water balance. Communication between endocrine cells and target tissues occurs via hormones released into the bloodstream and lymphatic system. Hematopoietic cells, endothelial cells, and most tissue types communicate by secreting cytokines that initiate a coordinated inflammatory response in neighbouring cells, tissues, and organs. Feedback loops regulate secretory pathways to ensure a correct physiological response.

Low plasma concentrations of a hormone usually stimulate synthesis and secretion by endocrine cells and vice-versa. For example, the main regulator of aldosterone production is the plasma concentration of angiotensin II, which increases during hypovolaemia.

Angiotensin II stimulates synthesis and secretion of aldosterone, and increased aldosterone concentrations lead to a restoration in blood volume. When the volume of blood increases, angiotensin II secretion drops, and thus aldosterone production ceases. Cytokines used in the brain's neuroendocrine axis have more complex signalling networks. Cross-talk between different signalling pathways leads to a positive or negative regulation of the synthetic chain of neurohormones. The neuropeptide vasopressin has been suggested to amplify the effects of circulating glucocorticoids during stress responses and to help sustain longer-lasting effects [101, 221, 231].

Chapter - 15

Plasma Biomarkers in Disease Diagnosis

The plasma proteome contains a wealth of information relevant for various diseases. Indeed, most of the most widely used plasma biomarkers are proteins. In this context, several plasma proteins, such as troponins (myocardial damage) or B-type natriuretic peptide (ventricular strain), are often closely associated with the same disease and can be considered together, but they are not normally encapsulated in a single panel. Many other proteins have been proposed as potential disease biomarkers, examining either their presence and concentrations or changes in glycosylation, but more comprehensive evaluations have shown that very few are truly specific to a particular disease. An important caveat is that many of these proteins are also produced in response to the presence of other diseases or stress not directly related to the disease for which they are being studied. The detection of excessive amounts of these proteins should thus be interpreted with care, adopting approaches based on the concept of cancer glycolysis for greater accuracy, especially when considering the sensitivity-specificity balance. Sensitivity assessment is equally crucial for potential biomarkers not seen in blood healthy controls; Careful measurements must be conducted because, despite decreased circulating levels, some proteins may still be detected with high sensitivity due to biomarker imbalances among different populations.

In addition to proteins, particular metabolite patterns associated with specific diseases are frequently detected in plasma. Most studies concentrate on the discovery of clusters of metabolites directly existing in blood; some integrate these with genetic expression data, but only a

few include profiling of protein activity as well. For example, the metabolic signature of colorectal cancer seems to be linked to the activity of various enzymes, the presence of diagnostic metabolites such as methylmalonate and the concentrations of other metabolites related to enzyme function. Metabolomics approaches can also be applied in prognosis, detection and monitoring of disease, such as the clear group separation obtained for lung cancer during its development. Biomarkers associated with psychiatric diseases are also among those most actively investigated; among these is the concentration of dimethylglycine and its possible implication in schizoaffective disorder. Additionally, the development of new methods for the detection of several compound classes, such as volatile organic compounds, that are not normally measured in complex media such as blood but are known to be involved in various diseases opens up new interpretations of plasma function, even during acute physiological alterations [232, 233, 234, 235].

Protein biomarkers

Changes in the concentrations of plasma proteins can provide important diagnostic information. Traditionally considered organspecific, the levels of certain proteins can be related to the functional status of a specific tissue or organ. Examples include aminotransferases and alkaline phosphatase, which are mainly associated with liver function; creatine kinase, with muscle; and lactate dehydrogenase, with various tissues, including liver and kidney. In most cases, however, the correlation is not that straightforward. It is often difficult to pinpoint the source of elevation or reduction in a given protein and relate it to any specific disease process. This is particularly true for the acute-phase reactants, the members of the coagulation and fibrinolytic systems, and the immunoglobulin classes.

The DNA or RNA associated with a particular disorder may or may not be of plasma origin. These nucleic acids can be taken advantage of in noninvasive detection tests. At present, pregnancy-associated factors are the only clinically established example of nucleic acid testing in

maternal plasma. Specifically, the detection of Y-chromosome signals (from the placental syncytiotrophoblast) in maternal plasma indicates the pregnancy is male. No other such testing has been validated for any other condition [236, 237, 238].

Metabolic biomarkers

comprise a large and heterogeneous class of disease indicators, arising from complex metabolic pathways affecting proteins, carbohydrates, lipids, etc. Profiling low-molecular-weight metabolites holds interest in many conditions, including diabetes, cancer, myocardial infarction, and more. Examples include indole-3-propionic acid, whose decrease correlates positively with ischaemic stroke severity, and 1,4-yourabetabin-5-pyrimidinemonophosphate, dysregulated in liquor of Alzheimer's patients. A hypothesis-free screening of blood plasma determined 66 metabolites connected with Alzheimer's, again confirming the link with aging and neurodegeneration. Plasma metabolomics additionally showed associations between altered tryptophan patterns and depression.

Diagnostic panels are constantly updated via in-depth validation of the strongest candidates. Metabolomes of lipoproteins separated into different density fractions enable deep analysis of HDL, LDL, IDL and VLDL metabolism. These concentrated lipoprotein-associated metabolites serve as biomarkers for dyslipidemia and cardiovascular disease risk profiling. Further panels hold diagnostic potential for Type 2 diabetes mellitus and heat-associated acute respiratory disease syndrome, and with added omics data, the use of plasma-derived nucleic acids as epigenetic and cancer markers is now feasible [239, 240, 241, 242].

Genetic and epigenetic markers

Detection of genetic and epigenetic changes in plasma-derived nucleic acids presents a promising avenue for disease risk assessment, prognostication, and monitoring, with special emphasis on cancer. Whole-exome and targeted sequencing of matched pairs of tumour and normal tissues has identified millions of somatic mutations in

thousands of cancer types, although only a small fraction of these changes overlap between different individuals. Thus, only a subset appear to be clinically useful as cancer-specific somatic DNA methylation markers for early detection and monitoring.

Detection of signatures of aberrant DNA methylation associated with a growing number of human diseases is possible because, unlike other modifications, DNA methylation markers can be detected as free DNA in blood plasma and other body fluids. Methylated-DNA immunoprecipitation and massively parallel sequencing (MeDIP-seq), combined with droplet digital PCR and methylation-specific PCR technology, allow for the precise cancer screening and detection of the early stages of tumourigenesis. Non-coding cell-free miRNAs can also be detected in blood plasma derived from cancer patients with various stages and types of lung, breast, and colorectal cancers. Specific miRNA fingerprints have been identified and associated with disease progression and tissue repair after lung injury [243, 244, 245, 246].

Clinical validation strategies

Clinical validation of plasma biomarker discovery encompasses three stages: analytical, clinical, and clinical utility validation. Analytical validation interrogates whether a biomarker assay reliably detects the suggested biomarker at its predicted concentration in a defined cohort without interference from biological matrix components, analytes, or other endogenous substances. It is often performed to comply with US Food and Drug Administration (FDA) guidelines for in vitro diagnostic medical devices. A variety of techniques have proven invaluable for analytical validation. Real-time polymerase chain reaction (PCR) techniques are routinely used for biomarker detection in plasma DNA or RNA samples. Mass Spectroscopy (MS) has become the gold-standard detection method for lipids, small metabolites, and drugs present at low concentrations, in addition to protein assays. Enzyme-linked immunosorbent assays (ELISAs) are commonly employed for protein quantification at various concentrations.

Biomarker progression through clinical validation usually relies on AGREE (A Guideline for Reporting Assessments of Diagnostic Evidence) criteria, which emphasize methodological strengths including cohort design, clinical context, and confirmation of the proposed diagnosis. Diagnoses predicted by the assays need to be confirmed through clinical guidelines. For predictive models, AGREE guidelines recommend a separate cohort for unbiased assessment of statistical performance because the development cohort is inherently inflated due to overfitting. The threshold model selection should rely on Bayesian metrics that allow balanced evaluation of discrimination and calibration, particularly in the context of low-prevalence diseases such as malignancies. These considerations are typically, but not exclusively, applied to proteomic-based predictive models when assessing biomarker progression through clinical validation [247, 248, 249, 250].

Chapter - 16

Plasma in Immunity and Inflammation

Components of the innate immune system are found in the plasma, which serve to protect the host from infection. The complement system consists of more than thirty plasma proteins, organized into three activation pathways (classic, lectin, alternative). These pathways lead to the production of potent inflammatory mediators involved in the anaphylactic response, opsonization of pathogens, and the formation of the membrane attack complex involved in the lysis of bacteria, fungi, and infected mammalian cells. Plasma also contains several acute-phase reactants (proteins, glycoproteins) whose synthesis and release by the liver is up-regulated during inflammation and infection. Apart from the enhancement of the pro-inflammatory response, some acute-phase reactants also have protective functions in the homeostasis of metal ion concentrations in plasma.

Primed T and B lymphocyte populations and their products (antibodies) mediate specific responses to pathogens. Also, monoclonal antibodies produced by hybridoma technology are widely used in medicine (serology, immunoassays), diagnostics (angioscanning, ALD-related diseases), and cancer immunotherapy. Diagnosis of HIV infection is accomplished by detecting antibodies against viral proteins in serological specimens, and the monitoring of HIV-dependent clinical status is accomplished by quantifying viral RNA in plasma [251, 252, 253, 254].

Innate immune components

Several innate immune components circulate in plasma, including

complements and acute-phase proteins. The complement system consists of more than 30 soluble proteins, most of which are synthesized in the liver. It is activated via three pathways: classical, lectin, and alternative. All pathways lead to the formation of a large enzymatic complex C5 convertase which cleaves C5 to generate C5a and C5b. C5a is a potent pro-inflammatory molecule that promotes term-8 leukocyte chemotaxis and activates mast cells. Complement activation directly eliminates the pathogen by opsonization, promoting phagocytosis, or by rupture through the membrane attack complex. Activation is tightly controlled by a range of specific soluble and membrane-bound complement regulators that help to prevent self-tissue damage.

Acute-Phase Proteins (APPs) are an important group of innate immune mediators. Their concentration in plasma changes substantially during infectious and inflammatory processes. Key APPs include C-reactive protein, serum amyloid A, and the members of the complement system. In humans, inflammatory signals such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and IL-1 induce their synthesis predominantly in the liver. Most APPs are believed to play a protective role in inflammation and tissue repair. Their concentrations can increase dramatically (>1,000%) in specific disease conditions, and analysis of APP levels has thus become an important clinical tool for diagnosis and prognosis across a range of diseases [255, 256, 257, 258].

Adaptive immune mediators

The adaptive immune system enables a specialized and highly effective response to new pathogens through the proliferation of antigen-specific lymphocytes. Circulating antibodies are essential to its function, neutralizing infectivity or promoting destruction through an array of proinflammatory effector mechanisms. Antigen-specific T lymphocytes provide help to antibody-forming B cells and have the ability to destroy infected cells directly. Both humoral and cellular arms of adaptive immunity remain poised for rapid reactivation in

response to previously encountered antigens, creating the hallmark property of immunological "memory." Beyond their primary role in host defense, an array of growth factors and other signals released during adaptive immune responses can alter organ functionality. Cytokine signaling thereby enables the host to adapt to immune insults while inducing further secretion of mediators that govern a breadth of biological processes.

Antibodies belong to a larger family of proteins known as immunoglobulins (Ig) that are produced by plasma cells (activated B lymphocytes). Antibodies are heterodimers composed of heavy and light chains, which are covalently joined by disulfide bonds. Polymorphism in the protein sequences of the heavy and light chains give rise to the incredible diversity in antibody specificity that enables each B cell to express high-affinity receptors for unique antigens. There are five major classes of antibodies IgM, IgG, IgA, IgE, and IgD distinguished from one another by the size and composition of their heavy chains, as well as by their effector functions. In the serum, IgM constitutes about 5 to 10% of the total antibody pool and is produced early in an immune response. IgG accounts for nearly 75% of serum antibody and is the predominant class during secondary immune responses. IgA is found in mucosal secretions, breast milk, and other external excretions. IgE is produced in low amounts but is responsible for mediating hypersensitivity reactions (allergies) through binding and activation of mast cells and basophils. Finally, IgD is expressed by mature B cells; its abundance in serum and biological function remains unclear [259, 260, 261, 262].

Acute-phase proteins

Are a family of proteins whose concentrations are modified during inflammation. They typically include C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin, α1-antichymotrypsin, and α1-acid glycoprotein, among many others. Their concentrations may change by a factor of five or more and are mediated by pro-inflammatory cytokines from activated macrophages, lymphocytes, and other

immunocompetent cells. Acute-phase proteins are used to support or confirm various diagnoses and assess prognoses; for example, high serum concentrations of CRP are associated with an increased risk of cardiovascular disease.

Acute-phase proteins follow the synthesis response to inflammation, trauma, malignancy, and myocardial infarction. While other acute-phase proteins may have diagnostic value, their decreases are often less useful than the increases of CRP, SAA, and albumin. The synthesis of CRP, SAA, transferrin, haptoglobin, and α1-acid glycoprotein is increased during inflammation, while the synthesis of albumin and transferrin is decreased. The synthesis of ceruloplasmin and α1-antichymotrypsin is modified at later stages and is influenced by the degree and duration of tissue injury or inflammation [263, 255, 264, 265].

Mechanisms of chronic inflammation

Markedly elevated plasma concentrations of cytokines, chemokines, and an array of other complex mix of soluble mediators are routinely found in patients with chronic inflammatory diseases, such as rheumatoid arthritis, lupus erythematosus, coronary artery disease, diabetes mellitus, and cancer. In many of these conditions, such changes in systemic levels of inflammatory molecules are not secondary manifestations of local tissue damage but indeed reflect an active role in perpetuating the disease. These circulating factors also have systemic consequences, and their sham use in rodents leads to murine models of the corresponding chronic inflammatory disorder. Systemic dysregulation of inflammation can therefore be seen as an additional form of advanced persistent inflammation that extends beyond the confines of local tissue homeostasis to impact the host as a whole. Pro- and anti-inflammatory signals can likewise circulate in pathological quantities and are viewed as both maladaptive and biologically significant in their own right.

Such alterations have crucial links with the virtues and perils of heterologous immunity defensive immune cross-reactivity against a

spectrum of pathogens that is mediated by a diverse repertoire of lymphocytes but that also engages missensed autoreactive-specificities associated with the risk of conducting detrimental disease in privileged sites of inflammation. Consistent with this view are the recent findings in COVID-19 patients, in which systemic inflammation and elevated levels of a broad range of pro-inflammatory cytokines, chemokines, and plasma markers of the neutrophil, monocytic, and haematopoietic response upon CD8+ T-cell activation predicted clinical evolution and prognosis. Both innate and adaptive immune processes seem to underlie the complex clinical courses observed, and sustained hyper inflammation of the respiratory tract was revealed to blind the immune response to co-infection and super infection [266, 267, 268, 269].

Chapter - 17

Therapeutic and Clinical Applications

Blood transfusion and exchange therapy are essential in managing life-threatening, volume-related derangements caused by trauma, surgical complications, and end-stage diseases. As the availability of well-characterized source material becomes limited, although rare blood groups can be harvested from volunteers, the preparation of large aliquots of ABO- and Rh-compatible products remains a challenge for most blood banks. Repeated exchanges or extensive plasma-product exposure can also pose risks of allergic reactions and transfusion-related acute lung injury. Therefore, cryopreserved, solvent/detergent-treated product pools, rich in immunoglobulins and coagulation factors, are often regarded as a safer alternative. Plasma preparations that are depleted of known, high-frequency antigenic determinants and transfused in combination with red cells are increasingly well tolerated by patients.

Numerous FDA-approved drugs for congenital or acquired diseases can be purified from commercial human plasma. Beyond immunoglobulin preparations, highly purified or recombinant forms of complement factors, coagulation factors, inhibitors to von Willebrand factor and protein C, coagulation factor concentrates, methemoglobin reductase, and a variety of lysosomal enzymes are readily available to clinicians. Virus inactivation removes the risk of transfusion-transmissible infections from blood-related plasma therapies. Furthermore, natural transpiring proteins are used for high-volume clinical development programs, while in-house methods provide the basis for the production of specific therapeutic agents [270, 271, 272, 273].

Plasma transfusion and exchange

Are techniques that utilize the therapeutic properties of both the liquid and cellular components of blood. Plasma transfusions are indicated to replenish essential protective or regulatory factors in patients whose concentrations are insufficient to restore physiological function. Since plasma components are distributed throughout the body tissue and fluids, substantial volumes may be absorbed by the blood compartment, and plasma transfusion not only augments concentrations but also helps maintain physiological homeostasis. In contrast, plasma exchange has the opposite effect on the recipient's plasma component temperatures and is designed to remove these factors from the patient while simultaneously restoring their concentration back to relatively normal ranges.

Transfusions of plasma during several clinical conditions prolong patients' survival, including severe burns, thrombocytopenic purpura, disseminated intravascular coagulation, severe acidosis, liver failure, digestion of blood by the pancreas, and hepatic cirrhosis associated with hypoproteinaemia. The primary plasma proteins responsible for these effects are albumin and coagulation factors. Albumin is important for maintaining plasma osmotic pressure and, when in short supply, infusion becomes necessary. Coagulation factor concentrations may also become critically low in liver disease, resulting in a bleeding diathesis that can be corrected quickly by fator replacement. However, because plasma is a complex liquid containing a multitude of proteins, hormonal factors, trace elements, electrolytes, and other components rozmally present in only small quantities, inappropriate use can be dangerous. Care must be taken to avoid hypervolemia and allergic reactions, and the selection of frozen-thawed rather than fresh-frozen plasma has been shown to minimize the risk of transfusion-associated lung injury. Plasma transfusion is therefore a powerful but dangerous therapeutic tool that requires thorough understanding and proper training.Severe systemic inflammatory response syndrome can occur in critically ill patients, and supportive therapy sometimes requires extracorporeal blood purification. A variety of processes, generically

termed "plasma exchange" or "blood purification", remove inflammatory mediators from the bulk plasma in contact with blood or hemodialysis membranes [274, 275, 276, 277].

Plasma-derived pharmaceuticals

Commercial plasma-derived pharmaceuticals draw upon therapeutic needs and the diversity and availability of plasma components. Intravenous polyvalent immunoglobulins, large-scale production of coagulation factors for hemophilia, and replacement therapy with aplasma protein deficiency are among the most common and broadly utilized. The ability to analyze and quantify most of the plasma components provides an essential guide for their matching with clinical needs. Precise targeting of the individual plasma components allows for the development of highly innovative therapeutic strategies ranging from oral preparations for autoimmune diseases to skin-substitutes and anticancer treatments.

Therapeutic applications of plasma-derived proteins include transfusion of Fresh-Frozen Plasma (FFP) units or plasma exchange in patients with specific antibody deficiency. Transfusion of FFP is also indicated for surgery patients requiring large-volume shedding or substantial Factor V deficiency. Plasma transfusion is also indicated for coagulopathy due to liver failure and Disseminated Intravascular Coagulation (DIC) and, more controversial, in patients with Systemic Inflammatory Response Syndrome (SIRS) or severe sepsis. Plasma-based compounds are also under development for orally administrable anti-inflammatory treatment. Other promising developments are the supplementation of natural-appearing skin substitutes in burn patients, the switch from allogeneic to autologous sources in skeletal surgery, and Intravenous Immunoglobulin G (IVIg) therapy in the deficit of immunological protection [278, 279, 280, 281].

Pharmacological interactions in plasma

Therapeutic agents exert metabolic effects by interacting with specific receptors located on target cells throughout the body. The

pharmacokinetics of orally administered drugs typically involve absorption into the blood plasma via the gastrointestinal tract and subsequent distribution to remote target tissues. The concentration of the active form of a drug at target tissues is determined by the drug's affinity for target tissue relative to blood plasma and by the volume of blood plasma that is in equilibrium with the drug in active tissues at the time of action.

Because blood plasma invariably contains many different molecules, molecules of competing drugs or endogenous metabolites may decrease the pharmacologic action of a drug by competing with it for binding and transport proteins. Blood plasma proteins especially albumin, alpha-acid glycoproteins, and alpha-1 globulins bind lipophilic drugs, and these plasma-protein-drug complexes serve as a source of sustained drug release into the free drug pool of plasma, reducing the area under the curve for drug drug concentration over time. For drugs that are heavily bound to plasma proteins, the unbound concentration determines the pharmacological action of the drug. Drugs that are highly dependent on the unbound fraction may be displaced from the proteins or alternatively may competitively bind to the drug-binding site on the proteins [282, 40, 76].

Personalized and precision medicine

Plasma profiling has emerged as a valuable tool for precision and personalized medicine. The concentration of various constituents varies with health status and individual profiles, making plasma suitable for discovery-driven exploration of both biomarkers and therapeutic targets. Applications include drug specificity prediction, disease prediction and prognosis in cancer and cardiology, celiac disease diagnosis, and even aging prediction. Concentration changes can have diagnostic utility and may also indicate physiological processes that predispose patients to future illness. Individualized risk assessment, including polygenic risk scores, may be complemented by plasma levels of specific metabolites and lipids.

Proteomics, metabolomics, and lipidomics allow the detection of

hundreds of proteins and metabolites in plasma samples. Multi-omcis data integration offers unprecedented opportunities to interrogate the biology of complex diseases. It capitalizes on combining powerful and complementary technologies to interrogate multiple biological layers at once, thus enabling a deeper and holistic understanding of disease. AI support will considerably help in data integration and clinical decision support. Next-generation biosensors with ultrahigh sensitivity and high specificity targeting important biomolecules in plasma will undoubtedly support future point-of-care applications for a variety of diseases. Multi-omics integration may also generate valuable insights for personalized or precision medicine [283, 284].

Chapter - 18

Future Directions in Plasma Research

A multi-omics systems biology approach is the best basis for an integrated view of plasma as a biochemical environment. A combination of proteomics, metabolomics and lipidomics offers the best picture of the plasma biochemical reaction medium. Alongside such methods, artificial intelligence can aid in plasma data integration and support diagnostic or therapeutic decisions. The next-generation biosensors of plasma analytes, analysed with the help of artificial intelligence, must integrate high sensitivity, specificity and applicability in near-patient or point-of-care settings. Artificial-intelligence-assisted tools allowing the connection of multi-omics plasma data with health- and disease-related phenotypes will contribute to a comprehensive view. Finally, a multi-omics approach to plasma analysis will facilitate the discovery of novel biomarkers and the development of innovative diagnostic tests and therapeutic strategies.

Omics integration will when properly executed create a detailed view of the complex world of plasma chemistry, pointing to integrated changes, imbalances and sudden events that affect the entire environment in a coordinated way. This approach allows the detection of causes and consequences of a single process in multiple changeable plasmalitomic-omic components (e.g. thalassemia; disorders of iron overload; certain hereditary lipid transport disorders; chronic inflammatory diseases such as Crohn's disease or ulcerative colitis, systemic lupus erythematosus, and rheumatoid arthritis).

Multi-omics integration (proteomics, metabolomics, lipidomics)

More sophisticated plasma analysis will integrate complementary information from multiple omics dimensions including proteomics, lipidomics, and metabolomics, thus contributing to a systems biology approach. Multi-omics approaches provide a systems-level understanding of biochemistry, enabling reconstruction of metabolic pathways and other complex networks within the cell. In this context, plasma is emerging as a key subject of interest due to its critical roles during health and disease.

Increasingly complex experimental designs are therefore facilitated by combining complementary analytical techniques to better describe the multifaceted nature of plasma. Plasma analysis represents a challenging application of metabolomic technologies, given its role as a complex biological fluid encompassing diverse molecular classes. To date, the plasma metabolome has been investigated using various approaches, but metabolomics remains in its infancy compared with metabolomic analyses of other biological tissues. Copious data exist regarding plasma proteomics and lipidomics, and these areas are now being integrated into a complete multi-omics platform [285, 92, 285, 92, 286].

Artificial intelligence in plasma analysis

Artificial Intelligence (AI) is revolutionizing research across diverse scientific domains and clinical applications. In plasma analysis, Machine Learning (ML) and Artificial Neural Networks (ANNs) are being applied to clinical laboratory data to reveal hidden relationships among measurements and health outcomes, enhancing decision support and stratified medicine by guiding therapeutic planning. In metabolomics, ML processes patterns in lipidomics, transcriptomics, or other complementary data sets to provide predictions with superior accuracy. Neural networks access vast arrays of genomic and plasmatic profiles, providing stratification for disparate health conditions. The marked increase in high-dimensional metabolic-associated data sets across several biological subjects has prompted the exploration of deep-learning neural networks as a feasible tool for processing such

data. ANNs, previously used mainly in medical-image analysis, are now being implemented for integration in various plasma-associated research arenas.

Artificial-intelligence-based approaches have been shown to accurately predict graft rejection using large metabolic, lipidomic, and transcriptomic data sets and to successfully address the age-related multifactorial geriatric syndrome issues for the four major components of metabolomics lipidomics, glycomics, and related pathways. AI-assisted decision making based on high-dimensional plasmalomic data will eventually augment the clinical decision-support process, and many studies thus strongly advocate a more-pronounced application of this technology for plasma research, clinical laboratory practice, and the medical literature.

Next-generation biosensors

Recent progress has advanced plasma diagnostics toward point-of-care application. Biosensors based on plasmonic nanostructures reveal metabolites, proteins, and nucleic acids at ultralow concentrations with high specificity. These systems enable simultaneous detection of multiple analytes associated with disease pathways.

Combine the principles of optical sensors, electrochemical detection, and bioelectronics. The core elements are optical biosensors based on Surface-Enhanced Raman Scattering (SERS) and Localized Surface Plasmon Resonance (LSPR). They exploit metal nanoparticles, which can amplify optical signals of target analytes enriched in the vicinity of their surfaces. As a result, these sensors demonstrate ultrahigh sensitivity. Analytes of interest include metabolites left in the bloodstream by tissue when they become hypoxic, pathogens, proteins, nucleic acids, and even small-molecule compounds in urine.

Three-dimensional nanostructured substrates increase the sensor surface-to-volume ratio, facilitate the adsorption of target samples, and significantly increase the SERS signal. By combining plasmonic nanostructures with enzymatic principles and intelligent design, it is

possible to produce “turn-on” sensors, in which the Raman signal is amplified only in the presence of a target analyte.

Innovations in plasma-based therapies

Emerging interdisciplinary research areas, including metabolic-stress-related liquid biopsies, the foetal environment, metabolomics-assisted digital twins of physiology, and atheroprotective diets, offer novel avenues to explore therapeutic options targeting unique alterations of plasma composition. Conversely, markers of dysregulated states of immunity, recovery and repair signalling, and disturbances of dependency and feedback-system layer connectedness may guide the refinement of plasma transfusions or concentrate-therapy approaches. In the field of biosciences and health-care support, the first associations of artificial intelligence with plasma-metabolome analyses also promise new breakthroughs.

Novel sensing platforms, incorporating advanced capabilities and fabricating techniques either as a stand-alone tool or integrated as an analytical module, will enable timely and specific diagnosis of a wide variety of diseases in a single analysis. Multimodal biosensors integrating optical and electrochemical transduction mechanism are gaining attention for their low detection limits for a variety of clinical biomarkers. Recently, a volumetric swelling-mediated frequency modulation of the sub-micrometre-cube resonators has achieved vapour detection down to 1 ppm at room temperature by swelling. There is a growing interest in miniaturized sensors suitable for portable and point-of-care testing [287, 288, 289, 290].

Chapter - 19

Conclusion

Modern research provides a comprehensive understanding of blood plasma that integrates insights from biology and chemistry. Biological studies have established the importance of plasma in maintaining homeostasis by transporting nutrients, wastes, hormones, gases, and other molecules throughout the body. Recent work has elucidated its chemistry, revealing the properties of blood plasma as an aqueous solution. Together, these perspectives permit a holistic view of blood plasma as a biochemical mixture, transport medium, and reaction milieu. The constituents of blood plasma interact chemically and biochemically, forming the basis for numerous systematic signaling and metabolic networks. An integrated approach from both chemistry and biology exposes the fundamental roles of blood plasma and paves the way for advances in biology and medicine.

The resulting understanding contributes to plasma-based clinical diagnostics, therapeutic applications, and future medical advances. Diagnostics based on protein biomarkers and metabolic panels allow disease detection and prognosis. Plasma transfusion as a treatment for trauma and liver failure, together with plasma-derived pharmaceuticals such as antibodies and clotting factors, has saved many lives. Plasma-derived therapeutics in transfusion medicine and hematology foster personalized medicine and precision health by linking genetic predisposition to altered plasma states. Personalized plasma profiling will guide rational therapy in multiple disease states, including cancer, cardiovascular diseases, metabolic disorders, and aging. Multi-omics integration will enhance systems biology applications with potential

for artificial intelligence-based decision support. New-generation biosensors will enable rapid and sensitive detection of multiple analytes. Emerging diagnostic, therapeutic, and translational avenues underscore the importance of blood plasma as a complex biofluid in health and disease.

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