REVIEW OF THE BASIC SCIENCES ASSOCIATED WITH APHERESIS

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Basic Sciences Presentation Goals

Knowledge as they apply to apheresis:

– Hematology
– Hemostasis (Coagulation)
– Immunology
– Immunohematology
– Laboratory Testing
HEMATOLOGY
Hematology

Basics of Blood
  – RBCs, WBCs, Platelets, Plasma

Hematology Tests
Hematology

Erythrocytes (Red Blood Cells)

– Produced in the bone marrow at the rate of two million per second
– Lifespan = 120 days
– Stored at 4-6°C for 35 or 42 days
– Primary function is transport of O2 and CO2 (hemoglobin).
Hematology

Hemoglobin

– Consists of iron-containing heme and globin chains
– $O_2$ and hemoglobin form oxyhemoglobin in lungs
– Hemoglobin releases $O_2$, which diffuses into tissues
– Deoxygenated hemoglobin picks up $CO_2$ as waste and carries it to the lungs where it is released and exhaled
Hematology

Leukocytes (White Blood Cells)
– The cells of the immune response
– Divided into three groups:
  • Granulocytes
  • Lymphocytes
  • Monocytes
Hematology

Leukocytes - Granulocytes

– Neutrophils (PMNS)
  • Primary function is phagocytosis

– Eosinophils
  • Allergic reactions
  • Parasitic infections

– Basophils
  • Allergic reactions
  • Produce heparin, histamine, serotonin
Leukocytes - Lymphocytes

- Generates the immune response
- Lifespan is three years or more
- Two types:
  - T- lymphocytes
  - B- lymphocytes
Hematology

Leukocytes - Monocytes

- Macrophages
  - Engulfs foreign matter
- Antigen - presenting cells (APC)
  - Introduces foreign antigens to T-lymphocytes for recognition and destruction
Platelets

- Produced in bone marrow by megakaryocytes
- Vital role in hemostasis
- Lifespan = 7 to 10 days
- Shelf life = 5 days
Hematology

Plasma

– Liquid portion of blood
– Consists of water, electrolytes, protein and metabolites
– Main plasma proteins:
  • Albumin
  • Globulin
  • Clotting factors
Hematology

Plasma Components

– Fresh Frozen Plasma
  • Plasma separated and stored at -18°C within 8hrs of collection
  • Contains all the coagulation factors

– Cryoprecipitate
  • Insoluble portion of thawed FFP
  • vonWillebrand factor, Factor VIII, XIII, fibrinogen
Hematology

Hematology Testing

– CBC
  • Hematocrit, Hemoglobin, WBC Count, Platelet Count
– Reticulocyte Count
  • Measures rate of erythropoiesis
– White Blood Cell Differential
  • Absolute White Count
– Red Cell Morphology
  • Anisocytosis
  • Poikilocytosis
Hematology

Red Cell Morphology

Normal
Hematology

Red Cell Morphology

Sickle Cell Disease
Hematology

Red Cell Morphology

Schistocytes
HEMOSTASIS
Hemostasis

Definition:
The process by which the body spontaneously stops bleeding and maintains blood in the fluid state within the vascular system.
Hemostasis

- Platelet function
- Coagulation factors
- Anticoagulation
Hemostasis

Process

– Platelets adhere to the site of injury
  • Von Willebrand factor (vWF) mediates plt adhesion

– Platelet plug formation
  • ADP stimulates platelet release
  • Platelet aggregation

– Initiation of clotting factors leading to formation of a fibrin clot
  • Platelet factor 3 (PF3) release
Hemostasis

Coagulation / Clotting Factors

– Produced by the liver
– Referred to by Roman numerals in the order in which they were discovered
– Interact in a cascade
Hemostasis

Coagulation / Clotting Factors

I Fibrinogen
II Prothrombin
III Tissue Thromboplastin
IV Calcium
V Labile Factor (Proaccelerin)
VI “No Function”
VII Stabile Factor (Proconvertin)

VIII Antihemophiliac Factor A (AHF)
IX Christmas Factor
X Stuart-Prower Factor
XI Plasma Thromboplastin Antecedent
XII Hageman Factor
XIII Fibrin Stabilizing Factor
Hemostasis

Coagulation Cascade

– Intrinsic pathway
  • Activated by contact with foreign surface, e.g. collagen
  • Screened by aPTT (activated partial thrombin time)
– Extrinsic pathway
  • Activated by tissue thromboplastin (Factor III)
  • Screened by PT (prothrombin time)
– Common Pathway
  • Coagulation factor activation shared by both pathways
  • Factor Xa, PF3, Ca++
**Hemostasis**

**Coagulation Cascade**

**Intrinsic Pathway**
- XII
- Prekallikrein
- XI
- IX
- IXa
- VIII
- Phospholipid
- Calcium

**Extrinsic Pathway**
- VII
- Tissue Factor
- Phospholipid
- Calcium

**Common Pathway X**
- Xa
- V
- Phospholipid
- Calcium

**Prothrombin** → **Xa** → **Thrombin**

**Fibrinogen** → **Fibrin**
Hemostasis

Fibrinolysis

– Dissolution of a fibrin clot in a healed vessel
– Restricts fibrin formation to the site of injury
– Components include:
  • Plasminogen
  • Tissue plasminogen activator (TPA)
– Results in fibrin degradation products
  • D-dimer assay
Hemostasis

Anticoagulation

– Heparin
  • Enhances plasma anti-thrombin III to prevent thrombosis
  • Prevents conversion of prothrombin to thrombin

– Coumadin
  • Blocks action of vitamin K
  • Factors II, VII, IX, X (vitamin K dependent)

– Citrate anticoagulants
  • Binds Ca++ to prevent coagulation cascade from going to completion
IMMUNOLOGY
Basic Concepts of the Immune System

- Immunity/Immune response
- Immunoglobulins
- Complement
Immunology

Immunity

– Natural Immunity
  • Conveyed at birth
  • Outside stimulus not required

– Acquired Immunity
  • Stimulated and developed after exposure to a foreign substance
Immunology

**Antigen**
- Foreign molecule capable of stimulating an immune response

**Antibody**
- Complex protein molecule produced by B cells/plasma cells in response to a foreign molecule.

**Immune Complex**
- Antigen/antibody complex
Immunology

T - Lymphocytes
- Cell-mediated Immunity
- Helper T-lymphocytes (CD-4+) activate B lymphocytes
- Suppressor/cytotoxic T-lymphocytes (CD-8+) destroy by cell lysis and responsible for immune response regulation

B - Lymphocytes
- Humoral Immunity
- Become plasma cells, which produce antibody
Immunology

IgM

- Largest immunoglobulin making up 7-10% of pool
- Predominant immunoglobulin in the initial phase of an immune response
- Mostly intravascular in plasma
- Binds complement
Immunology

IgG

- Makes up the greatest number of immunoglobulin; 70 -75 %
- Predominant antibody in second exposure to antigen
- About 50 % extravascular
- Four subclasses: IgG₁, IgG₂, IgG₃, IgG₄
- Small enough to cross the placenta
Immunology

IgA
- Makes up 15-20% of pool
- Function prevents access of foreign substances into body

IgE
- Small quantities in plasma
- Allergic reactions

IgD
- No known function
Immunology

Complement

- System of 20-30 different proteins
- Activation triggered by immune complex or foreign antigen
- Complement activation leads to cell lysis
  - Classical Pathway
  - Alternate Pathway
Immunology

Primary Immune Response

– Response after first encounter with foreign antigen.
– Lag phase is about 1 week
– Primary antibody formed is IgM
– Typically followed by IgG
Secondary Immune Response

- Second exposure to the same antigen
- IgM and IgG appear at the same time with marked increase of IgG levels
- IgG is the predominant antibody.
IMMUNOHEMATOLOGY
Immunohematology

- Human Leukocyte Antigen (HLA) System
- ABO, Rh antigen and antibody
- Donor Compatibility
Immunohematology

HLA System

– Human Leukocyte Antigens
  • Found on cell membranes of all body cells except for red blood cells
  • Major histocompatibility complex (MHC) on chromosome 6 codes for the antigens of the HLA system.
Immunohematology

HLA System

– Class I Antigens
  • HLA A, B, C
  • Important in rejection after tissue transplantation

– Class II Antigens
  • HLA DR, DQ, DP
  • Important in recognizing non-self HLA antigens

– Each individual inherits two HLA-A, two HLA-B antigens and so forth.
Immunohematology

HLA System

– Each locus is designated by a different letter followed by a number:
  • A1,2, B1, 28
– Splits are antigens that share common antigen determinants (epitopes)
  • B44 (12), B45(12)
  • Each reacts with anti-HLA-B12
– Cross Reactive Groups (CREGS)
  • Antibodies to shared determinants result in cross-reaction
  • e.g. A1 cross-reacts with A3, A11, A6
HLA System

- Donor Selection
  - Platelet Transfusions
    - Loci A and B
    - Splits and cross-reactive antigens better than mismatched antigens
  - Bone Marrow/Stem Cell Transplants
    - Loci A, B, C, DR and DQ
ABO Blood Group

– The most important blood group system in terms of transfusion therapy.
– The ABO locus is on chromosome 9.
– Antigens are located on the surface of red blood cells and platelets.
Immunohematology

ABO Blood Group

PRECURSOR CHAIN

H

O

L-FRUCOSE
ABO Blood Group

PRECURSOR CHAIN

N-ACETYL GALACTOSAMINE

A
Immunohematology

ABO Blood Group

PRECURSOR CHAIN

D-GALACTOSE

B
ABO Antibodies

– Antibodies to the ABO antigens are naturally produced after birth
– Located in intravascular space
– IgM is the predominant immunoglobulin class
– Capable of intravascular hemolysis and complement activation.
Immunohematology

ABO Antigen/Antibody Relationship

<table>
<thead>
<tr>
<th>Red blood cell type</th>
<th>Group A</th>
<th>Group B</th>
<th>Group AB</th>
<th>Group O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibodies present</td>
<td>Anti-B</td>
<td>Anti-A</td>
<td>None</td>
<td>Anti-A and Anti-B</td>
</tr>
<tr>
<td>Antigens present</td>
<td>A antigen</td>
<td>B antigen</td>
<td>A and B antigens</td>
<td>None</td>
</tr>
</tbody>
</table>
Immunohematology

Red Blood Cell Compatibility

A → O → B
A → O → AB
A → O → B
A → O → AB
Immunohematology

Plasma Compatibility

![Diagram showing plasma compatibility types: A, B, AB, O.]}
Rh Antigen

- Located on red cells only
- Five alleles: D, C, E, c, e
- The presence or absence of the Rh ("D") antigen on red cells determines whether a person is Rh positive or Rh negative
- 85% Rh positive, 15% Rh negative
Rh Antibody

- The “Rh” antibody is NOT naturally occurring
- Develops as result of transfusion or pregnancy
- IgG antibody; crosses placenta
Immunohematology

Rh Compatibility

– Rh positive or negative blood may be transfused to an Rh positive patient

– Rh Negative patients should receive only Rh negative blood.
LABORATORY TESTING
Laboratory Testing

Coagulation/Hemostasis

- PT
- aPTT
- Fibrinogen
- D-dimer
Laboratory Testing

Protein Electrophoresis

– The movement of colloidal particles in an electric field

– Detection and quantitation of hemoglobin
  • HbA, HbF, HbS, HbC

– Detect or differentiate hypergammaglobulinemia
  • e.g. Waldenstroms, multiple myeloma,
Laboratory Testing

Chemistry
- LDH
- BUN
- Creatinine
- Electrolytes
- Total Protein
Laboratory Testing

**HLA**
- Antigen and antibody detection
- Crossmatch

**Blood Bank Tests**
- ABO group Rh type, antibody screen, crossmatch
Laboratory Testing

Infectious Disease Testing (donor eval.)

- HIV
- Hepatitis B
- Hepatitis C
- HTLV
- Syphilis
- Chagas Disease
- West Nile Virus
THANK YOU