The Red Cell Membrane: structure and pathologies

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The Red Cell Membrane

- Structure
- Maintenance of shape
- Pathology
- Interaction with malaria
• RBC is essentially a ‘bag of haemoglobin’
• The RBC is unique amongst eukaryotic cells in that it is anuclear, has no cytoplasmic structures/organelles.
• Structural properties are linked to the membrane.
• An 8 micron cell needs to be able to deform to pass through 3 micron capillaries/reticuloendothelial system without fragmentation.
• **Structure of RBC membrane:**
  – Lipid bilayer (40%)
  – Membrane proteins (52%)
  – Carbohydrate (8%)

• **Planes of design:**
  – ‘Vertical interaction’
    • Stabilise the lipid bilayer membrane
  – ‘Horizontal interaction’
    • Support structural integrity of RBC.
Structure of RBC membrane
Membrane Lipids

- Asymmetric phospholipid distribution
- Unesterified, free cholesterol between layers.
- Choline containing, uncharged phospholipids, outer layer:
  - Phosphatidyl choline (PC) (30%)
  - Sphingomyelin (SM) (25%)
- Charged phospholipids, inner layer:
  - Phosphatidyl ethanolamine (PE) (28%)
  - Phosphatidyl serine (PS) (14%)
Membrane Lipids

• Asymmetric phospholipid distribution is maintained by:
  – Differential rate of diffusion through membrane bilayer of choline containing phospholipids (PC and SM diffuse slowly)
  – Charged phospholipids interaction with membrane skeletal protein.
  – Active transport of aminophospholipids (PC and PE) from outer to inner layer.
Membrane Lipids

- Unesterified cholesterol lies between the two layers of the lipid bilayer.
- Mature RBC cannot synthesize lipids in vivo: renewal through exchange between plasma and membrane lipids.
- PS on the outer layer may be recognized by macrophages as a signal for attachment and phagocytosis.
  - May occur following RBC damage (e.g., b thalassoc RBC scrambling due to oxidative damage).
Membrane Proteins

• Integral proteins
  – Embedded in membrane via hydrophobic interactions with lipids.

• Peripheral proteins
  – Located on cytoplasmic surface of lipid bilayer, constitute membrane skeleton.
  – Anchored via integral proteins
  – Responsible for membrane elasticity and stability.
Integral Proteins

- Band 3
- Glycophorin
- Aquaporin
Band 3

• Constitutes 25% of total membrane protein
  – 911 Amino Acid protein
  – Comprises 3 distinct proteins:
    • Cytoplasmic domain:
      – Hydrophilic, interacts with proteins of skeleton
    • Transmembrane domain
      – Contains multiple membrane spanning domains
      – Forms the anion transporter
    • C-terminal domain
      – ?binds carbonic anhydrase
  – Single oligosaccharide possessing blood group antigen I and i.
Band 3

- Functions:
  - Anion transport
    - Exchanges bicarbonate for chloride
  - Structural:
    - Linkage of lipid bilayer to underlying membrane skeleton.
      - Interaction with ankyrin and protein 4.2, secondarily through binding to protein 4.1.
    - Important for prevention of surface loss.

- Chromosome 17
Glycophorins

- Comprise 2% of RBC membrane proteins.
  - Sialic acid rich glycoproteins (A,B,C,D)
- 3 domains:
  - Cytoplasmic
  - Transmembrane: single spanning alpha helix
  - Extracellular: glycosylated
- Imparts a negative charge to the cell, reducing interaction with other cells/endothelium.
- Glycophorin A carry M/N, Gerbich blood group specificities.
- Glycophorin C/ Protein 4.1/ p55 complex, and Glycophorin A, appear important for P falciparum invasion of and development in RBC.
Aquaporin 1

- Selective pores for water transport
- Allow RBC to remain in osmotic equilibrium with extracellular fluid.
Rh Group Antigens

- Carried by non-glycosylated membrane proteins.
Peripheral Membrane Proteins

- Spectrin
- Actin
- Protein 4.1
- Pallidin (band 4.2)
- Ankyrin
- Adducin
- Tropomycin
- Tropomodulin
Red cell membrane skeleton

- Basic unit is a hexagonal lattice with 6 spectrin molecules.
- Each linked to the next complex by multiple spectrin tetramers.
- Regular organisation of spectrin/actin/4.1 complexes.
- Ankyrin links the lipid bilayer to membrane skeleton via interaction with band 3.

The spectrin molecules form a mesh-like pattern that is anchored to the membrane by ankyrin molecules (ellipses). The basic shape is hexagonal (shaded).
Spectrin

- Flexible, rod like molecule, 100nm length.
- Responsible for biconcave shape of RBC
- Two subunits:
  - Alpha and beta, entwined to form dimers.
  - Associate head to head to form tetramers
  - Excess alpha subunits produced, beta subunits at rate limiting quantity.
    - Single beta allele deficiency causes disease
    - Need both alpha alleles damaged to cause disease.
Spectrin

• Beta spectrin:
  – Attachment for ankyrin near C terminus (which binds cytoplasmic tail of band 3) thus attachment of skeleton to lipid bilayer.
  – At N terminus:
    • Attachment for 4.1 protein (assoc with glyophorin C) – second anchor point with lipid membrane.
    • Binding sites for actin filaments and protein 4.1 – forming a junctional complex.
Actin

- Short, uniform filaments 35nm in length.
- Length modulated by tropomyosin/tropomodulin.
- Spectrin tail associated with actin filaments.
- Approx 6 spectrin ends interface with one actin filament, stabilised by protein 4.1
Other Peripheral Proteins

- **Protein 4.1**
  - Stabilises actin-spectrin interactions

- **Adducin**
  - Also stabilises interaction of spectrin with actin.
  - Influenced by calmodulin
  - (thus can promote spectrin-actin interactions as regulated by intracellular Ca concentration)
Ankyrin

- Interacts with band 3 and spectrin to achieve linkage between bilayer and skeleton.
- Augmented by protein 4.2.
Red Cell Mechanics

• Deformability is an important property of red cell function.

• Influenced by:
  – Cell shape (ratio of cell surface area to cell volume)
  – Cytoplasmic viscosity (regulated by MCHC and thus cell volume)
  – Membrane deformability and stability
Cell Shape

• Biconcave disc shape creates an advantageous surface area/ volume relationship.

• Facilitates deformation whilst maintaining constant surface area.
  – Eg RBC of volume 90fL has SA 140sq.microns
  – If a sphere: 90fL would have SA 98sq.microns.
Cell shape

- Reticulocytes contain mitochondria and ribosomes, and can synthesise Hb and some membrane components, have very limited deformability (10% of mature RBC).
- Progressive loss of intracellular and membrane components results in biconcave shape and improved deformability.
Cell shape

- SA/V ratio alterations will result in more spherical shape with less redundant surface area, and thus less capacity for deformability and diminished survival.
  - Membrane loss = reduced SA;
  - Increase in cell water content = inc Vol.
Cytoplasmic Viscosity

- Determined by MCHC, and thus by cell water content (managed by integral proteins).
- As MCHC rises, viscosity rises exponentially.
- Cell dehydration, due to failure of transport processes due to intrinsic problems with transport proteins (hereditary xerocytosis) or interactions with abnormal Hb (esp sickle cell, b thal).
Membrane Deformability/ Stability

• During pressure upon RBC, spectrin molecules undergo reversible change in conformation: some uncoiled and extended, others compressed and folded.
• During extreme or sustained pressure, membrane exhibits permanent “plastic” deformation.
• Deformability can be reduced by increases in associations between skeletal proteins or between skeletal and integral (esp band 3) proteins.
Pathology

• Vertical Interactions
  – Hereditary Spherocytosis

• Horizontal Interactions
  – Hereditary elliptocytosis
  – South East Asian Ovalocytosis

• Cholesterol content:
  – Acanthocytosis

• Hydration:
  – Stomatocytosis/ xerocytosis
Hereditary Spherocytosis

- Requires an RBC membrane defect +
- An intact spleen.
Hereditary Spherocytosis

- Molecular pathology is heterogenous:
  - Isolated deficiency of spectrin
  - Combined deficiency spectrin and ankyrin
  - Deficiency of band 3 protein
  - Deficiency of protein 4.2
- Weakening of vertical contacts between skeleton and overlying lipid membrane/ integral proteins.
- Consequent destabilisation of bilayer membrane, loss of lipid from membrane, reduction in SA: spherocytosis.
- Reduced deformability: entrapment in the spleen (fenestrations in splenic sinuses 2-3mcm diameter).
  - Removal
  - Further damage, reduction in membrane and changes in SA/V
• hereditary spherocytosis is a deficiency of membrane surface area
• Defects of band 3, spectrin, ankyrin, or protein 4.2 lead to destabilisation of the overlying lipid bilayer and release of lipid in microvesicles.
• Reduction in SA and subsequent spherocytosis.
HS

- Deficiency of ankyrin
  - Commonest abnormality in HS.
  - Autosomal dominant

- Isolated spectrin deficiency:
  - Beta spectrin mutations dominantly inherited.
    - Usually private point mutations resulting in reduced mRNA accumulation. Involve site of linkage to actin
  - Alpha spectrin mutations recessive
    - Have severe phenotypes (as absent alpha spectrin produced).

- Deficiency Protein 4.2:
  - Recessively inherited.
  - Japanese.
Hereditary Elliptocytosis

• Common HE:
  – Dominant
  – Highly variable severity.
  – Severe end: Hereditary Pyropoikilocytosis
    • Numerous microspherocytes, fragments, poikilocytosis
  – Mechanism: defect of or near the spectrin heterodimer self association site.
  – Results in disruption of hexagonal lattice.

• Haemolytic Ovalocytosis

• South East Asian Ovalocytosis
Common Hereditary Elliptocytosis

- **Spectrin mutations:**
  - Commonest defect (2/3).
  - Mutations can reside on either alpha or beta chain
  - Disrupt self association of dimers into tetramers.
  - Impairs two dimensional integrity of skeleton.

- **Protein 4.1 mutations:**
  - Impaired binding of spectrin to actin.
  - Partial deficiency: mild. Complete deficiency – severe haemolytic anaemia

- **Glycophorin C deficiency:**
  - Heterozygous are asymptomatic, homoz – mild anaemia
  - Partial deficiency in 4.1 also.
  - Precise pathobiology unclear.
Common Elliptocytosis

• Mechanism of elliptical shape is unclear.
• Precursors are round. Deformation in microcirculation/ prolonged shear stress may render HE RBCs elliptical.
HE

• Molecular basis of severity:
  – Spectrin content of cells.
  – % of dimeric spectrin
    • Degree of dysfunction of spectrin.
    • Proximity of mutation in spectrin to spectrin-actin association.
    • Gene dose (hetero vs homozygous vs double heterozygote)
SE Asian Ovalocytosis

• Highly resistant to invasion by malaria.
• Mutation in band 3:
  – Heterozygote for 2 mutations:
    • Deletion of 9 codons at boundary cytoplasmic/ membrane domains.
    • 56 Gly to Lu substitution (band 3 memphis)
  – Tighter binding of band 3 to ankyrin, inability to transport sulfate anions, increased TK phosphorylation of band 3 protein.
  – Results in red cell with increased rigidity
• Homozygote mutations lethal.
SAO
Acanthocytosis

• Spur cell haemolysis of severe liver disease:
  – Usually, free cholesterol in membrane readily equilibrates with plasma cholesterol (cf esterified cholesterol).
  – In CLD, abnormal lipoproteins with free cholesterol/phospholipid ratio.
  – Free chol partitions deposits onto RBC membrane. Increase in free cholesterol.
  – Rx: theoretically splenectomy. Really, Rx CLD.
• Stomatocytosis
  – Molecular basis unclear - ?loss of integral protein ‘stomatin’ (7.2)
  – Increased Na and H2O intracellular content; mild decr K.
  – Excess activation Na/K ATPase unable to compensate.
• Xerocytosis:
  – Group of AD haemolytic disorders characterised by cell dehydration.
Genetic resistance to Malaria

- Vector lifecycle
- Human lifecycle:
  - RBC
  - hepatic
• Genetic resistance to malarial infection resides in the red cell.
  – Impairment of merozoite entry to red cell
  – Impairment of erythrocyte growth
  – Prevention of erythrocyte lysis and release of merozoites into bloodstream.
Invasion of RBCs

• Incompletely understood
Invasion of RBCs
• DBL (Duffy binding like) proteins
  – P Vivax enters RBC via Duffy antigen.
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  – Via Duffy binding like (DBL) proteins localised to the apical end of merozoites.
  – Duffy is absent amongst many africans, arabs, jewish.
• Similar protein between P Falcip and glycophorin.
• RBL (Reticulocyte binding like) proteins
• Falcip infected RBCs:
  – Coated in adhesive ‘knobs’.
  – *P. falciparum* erythrocyte membrane protein 1 (pFEMP1) expressed at RBC surface:
    • Can recognise various DBL RBC receptors on parasites
• Glycophorins:
  – Heterogeneity between P Falciparum strains and binding to RBC.
  – DBL EBA 175 binds to glycophorin A.
  – Gerbich negative phenotype appears to protect against P Falciparum and P Vivax infection (PNG).
• SAO:
  – Resistance to parasitaemia and cerebral malaria (P Falcip, Vivax, and Knowlesi).
  – Likely distal to entry, reduced entry
  – Malarial infection seems to increase the % of ovalocytes.
• HE:
  – Resistance to malaria invasion by P Knowlesi and Falciparum
  – ? Malaria needs protein 4.1/glycophorin C/ p55 complex for parasite invasion and development.
  – Mature parasites in RBCs generate MESA (Mature parasite infected erythrocyte surface antigen), which binds to protein 4.1 normally. Accumulates in RBC without 4.1 -> impairs parasite development.

• HS:
  – Role in protection vs malaria is uncertain.
• G6PD
  – Strong epidemiologic evidence of association with malarious environments.
  – Mechanism of protection remains unclear.
  – Impaired parasite growth; better phagocytosis of infected RBC at earlier stage of maturation.
• Thalassaemias:
• Risk of severe malaria lower in alpha thal -/-+/- and +-/++.
  – Mechanism of protection unclear:
    • Bind more Ab per SA infected RBC than control cells
    • Increased vivax infection in youth -> ?cross immunity to Falcip?
    • Reduced rosette production (assoc with cerebral malaria).
• **Sickle Cell:**
  – Infected RBCs sickle and are removed from circulation.
  – Sickled cells at low O2 tension inhibit parasite survival.