

Immunological aspects of haematological disorders

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Learning outcomes

- Understand immune mediated red blood cells disorders.
- Understand immune mediated platelets and clotting system disorders.
- Understand immune mediated leukocyte disorders.

Immune mediated disorders of RBCs

- Immune mediated haemolytic anaemia (IMHA).
- Acquired aplastic anaemia (AA) and pure red cell aplasia.
- Paroxysmal nocturnal haemoglobinuria (PNH).

Immune haemolytic anaemia

- IMHA is characterized by immune destruction of red blood cells that have been coated with immunoglobulins, complement, or both, resulting in direct destruction or phagocytosis and removal from systemic circulation.
- The immune haemolytic anaemias are distinguished from the non-immune by detecting antibody on the surface of RBCs by the direct antiglobulin test (DAT).

Immune haemolytic anaemia

- Antibody-mediated haemolysis either caused by autoantibodies or alloantibodies.
- Accordingly, IMHA can be classified into autoimmune haemolytic anaemia and alloimmune haemolytic anaemia.

Autoimmune haemolytic anaemia

- Autoimmune haemolytic anaemia (AIHA) is characterized by a positive Coombs test or DAT, which detects antibody, with or without complement, on the red blood cell surface.
- AIHA is classified into warm and cold types depending on the characteristic temperature at which the red cell autoantibody is mostly reactive.
- Haemolysis is mainly extravascular.
- Could be primary or secondary.

Antigen type	Antibody	Diseases	Associations
Autoimmune	Warm antibody	Primary Secondary	Idiopathic Autoimmune diseases (ITP, SLE, Rheumatoid arthritis) Lymphoproliferative disorders Infections (EBV) Ovarian cysts Ovarian carcinoma and some other cancers Drugs
	Cold antibody	Cold haemagglutinin disease (CHAD) Cold antibody syndromes	Infections (<i>M. pneumoniae</i>), lymphoproliferative disorders
	Donath–Landsteiner antibody	Paroxysmal cold haemoglobinuria (PCH)	Post viral, syphilis
Alloimmune	Induced by red cell antigens	Haemolytic transfusion reactions Haemolytic disease of the newborn (HDN) Post-stem-cell allografts	
	Drug dependent	Antibody/macrophage mediated Antibody/complement mediated Membrane modification Autoimmune	

Immuneopathogenesis

- Resembles type II immune reactions.
- There is disturbance of the immune system in which T-lymphocyte control of autoreactive B lymphocyte clones is reduced.
- Autoantibodies could be IgG or IgM and rarely IgA.
- Autoantibodies are not specific and show reactivity against a number of antigens on RBCs.

Immunopathogenesis

Warm-acting antibodies:

- Active in vitro at 37 °C.
- Polyclonal and predominantly IgG antibodies.
- Target antigens: most commonly in the Rh blood group complex, less commonly either anti-e, anti-D or anti-c.
- Patterns detected by the DAT on the RBCs surface are as follows: IgG alone, IgG and complement, and complement alone.

Immuneopathogenesis

Cold-acting antibodies:

- Predominantly IgM: can be monoclonal or polyclonal.
- Most actively bound to antigen in the cold (4 C).
- Target antigens: there is specificity for anti-I, rarely for anti-Pr, anti-P, anti-M or anti-N and even cold reacting anti-A or anti-B.
- Act both as agglutinins and lysins in vitro.

Immuneopathogenesis

- Mechanisms for immune red cell destruction:
 1. Cell-mediated extravascular immune destruction: carried out by macrophages of RES through their Fc or complement receptors.
 2. Complement-mediated intravascular haemolysis: a minor mechanism for red cell destruction in AIHA.

Immunopathogenesis

- The spleen is the major site of RBCs destruction when IgG alone is the main Fc-binding protein on the RBCs surface.
- While the liver is the major site for trapping and phagocytosing C3b-coated red cells: the liver sinusoids contain the largest concentration of C3b-binding macrophages.
- Macrophage destruction of red cells is the main mechanism of haemolysis in IgM cold-antibody-coated cells.

Autoimmune haemolytic anaemia

Diagnosis:

- Coombs' test: identifies the presence of immunoglobulin and C3 on the red cell membrane.
- Assays are done at different temperatures to identify warm or cold antibodies.
- Direct test: an anti-human IgG is used to demonstrate pre-bound IgG on the patient's red cells.
- Indirect test: the patient's serum is incubated with normal erythrocytes to demonstrate the presence of anti-red cell antibodies in the serum.
- Treatment: treat underlying disorder, steroids.

Acquired aplastic anaemia

- Aplastic anaemia (AA), a rare bone marrow failure syndrome (BMFS), is defined by pancytopenia with a hypocellular bone marrow in the absence of an abnormal infiltrate and with no increase in reticulin.
- Acquired AA is most often idiopathic, though sometimes a drug or chemical or a virus infection is implicated.

Immunopathogenesis

- Acquired aplastic anaemia is an autoimmune disorder in which there is an aberrant immune attack, probably by CD8 cytotoxic T cells directed against the haemopoietic stem cell.
- In acquired AA, it is proposed that an initiating event, such as a virus or drug, provokes an aberrant immune response.
- The result is triggering oligoclonal expansion of cytotoxic T cells that destroy haemopoietic stem cells.

Immunopathogenesis

- It is unclear why T cells are activated in AA.
- HLA genes may play a role: HLA-DR2 alleles and some class I HLA-B and HLA-A alleles are overrepresented in AA, indicating a possible role for antigen recognition.
- The specific cytotoxic T-cell targets on haemopoietic stem and progenitor cells have not been identified.

Immune pathogenesis

- On the molecular level, genetic profiling of bone marrow cells (CD34+ cells) shows upregulation of genes related to the immune response, e.g genes for cytokines and cytokine receptors and signal transduction genes, apoptosis genes and downregulation of some antiapoptotic genes- thus enhancing the immune response against BM cells.

Immune pathogenesis

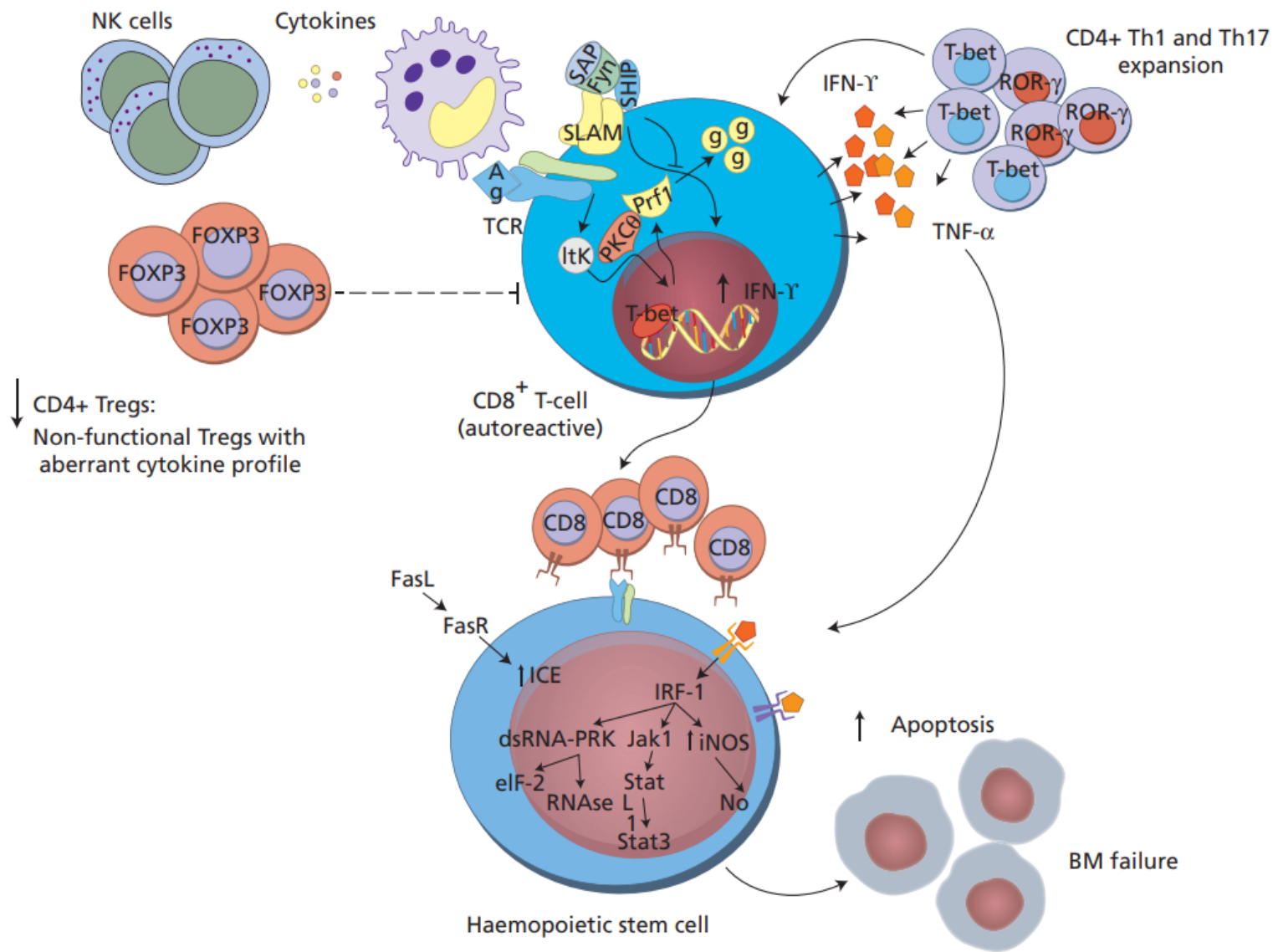
- A defect in the glycosylphosphatidylinositol (GPI) anchor may be the trigger for the immune response against normal progenitor cells through aberrant expression of intracellular GPI protein(s) while at the same time providing a protective mechanism for GPI-defective cells.
- 10–33% of AA may develop PNH.

Immunopathogenesis

- Strong evidence that AA has an autoimmune nature is based on the following observations:
 1. Haematological recovery after IST with antithymocyte globulin (ATG) and ciclosporin (CSA) occurs in the majority of patients.
 2. There are activated autoreactive oligoclonal CD8+ T cells present in blood and bone marrow that release IFN- γ and TNF- α , cytokines that inhibit haemopoiesis.
 3. Intracellular IFN- γ levels in T cells correlate with response to IST.

Immunopathogenesis

4. Increased Fas expression on bone marrow CD34+ cells indicates increased apoptosis.
5. T cell repertoire analysis shows oligoclonal expansion of CD8+ T cells in AA, MDS and PNH.
6. Transcription factor T-bet is upregulated resulting in increased expression of IFN- γ .
7. CD4+CD25+FOXP3+ regulatory T cells are impaired and clonally restricted Th1 are expanded inducing an inflammatory microenvironment.



Pure red cell aplasia

- Causes are similar to those of aplastic anaemia but limited to red cell lineage.
- Parvovirus B19 can cause prolonged red cell aplasia in the immunosuppressed (eg. HIV).
- Autoantibodies to red cell precursors can be detected.
- T-cell responses and inhibitory NK cells have also been demonstrated.
- It may respond to treatment with

Paroxysmal nocturnal haemoglobinuria (PNH)

- PNH is unique as an acquired haemolytic disorder in which the defect is intrinsic to the red cell.
- It is due to an acquired (clonal) deficiency of complement control proteins on the red cell surface.
- Patients have a propensity to develop thrombosis.
- PNH can also be associated with bone marrow failure.

Pathophysiology

- PNH results from the expansion of an abnormal haemopoietic clone that arises following an acquired mutation in PIG-A gene.
- This gene is critical for the biosynthesis of glycosylphosphatidylinositol (GPI) structures- mutations leads to deficiency in GPI synthesis.
- GPI molecules are membrane anchors to which large number of cell surface antigens are attached to the cell

Pathophysiology

- GPI-deficient clones only expand if there is an additional factor that encourages their selection e.g a second genetic event.
- This is indicated by the association between PNH and other bone marrow failure syndromes.
- Affected molecules of red cells include: CD55, CD59, and HRF65- C8-binding protein.
- These molecules prevent accidental lysis of red cells by complement.

Pathophysiology

- PNH clones are detectable in up to 50% of patients with aplastic anaemia.
- This suggests that such bone marrow failure syndromes are permissive for the expansion of PNH clones.
- It is presumed that PNH stem cells evade the autoimmune attack against haematopoietic cells seen in AA due to GPI deficiency.

Pathophysiology

This immune escape seen in AA with PNH clones implies:

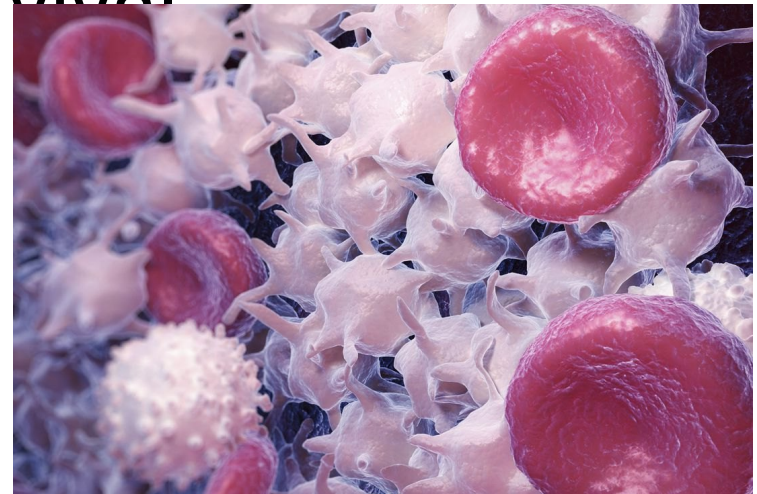
- Either the immune attack in bone marrow failure syndrome is, at least in part, directed through a GPI-linked structure.
- Or that the biology of the PNH stem cell is abnormal, protecting it from immune-mediated attack.

Paroxysmal nocturnal haemoglobinuria (PNH)

- The clinical picture in PNH depends on the balance between three components: intravascular haemolysis, bone marrow failure and thrombotic complications.
- Red cells tend to lyse at night when the pH of serum drops in the absence of the relevant surface molecules (CD55, CD59).
- Eculizumab has revolutionized PNH treatment - a monoclonal antibody that binds to C5 and prevents cleavage by the C5 convertase, blocks complement activation and prevents haemolysis.

Primary immune thrombocytopenia

- Primary immune thrombocytopenia (ITP) is an autoimmune bleeding disorder that affects both children and adults and is characterized by thrombocytopenia, with increased marrow megakaryocytes, and shortened platelet survival



Primary immune thrombocytopenia

- ITP is an autoantibody disorder in which platelets, opsonized with antiplatelet antibody are removed prematurely by the RES along with relative platelet underproduction which contributes to the thrombocytopenia.

Pathophysiology

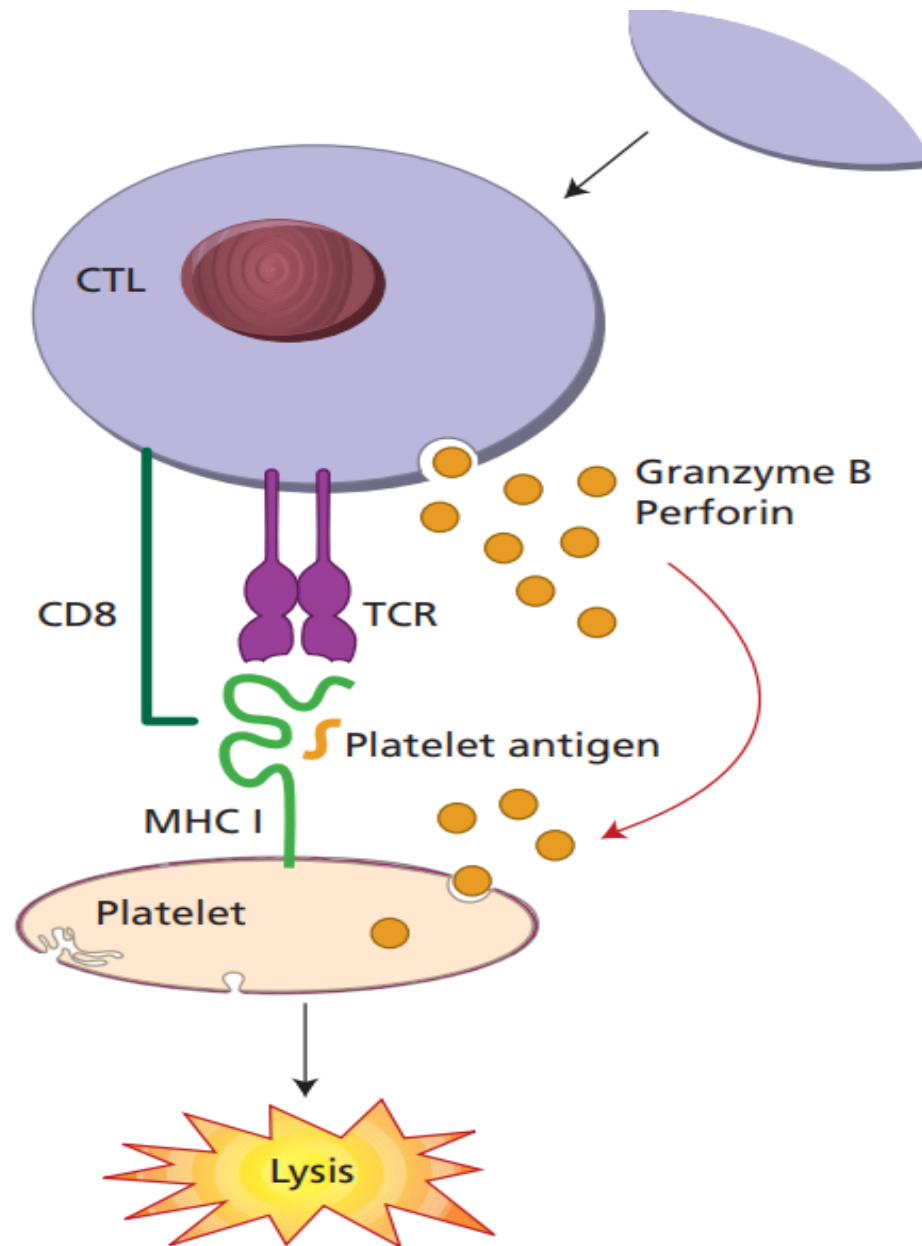
- ITP in adults is a typical organ-specific autoimmune disease.
- As other AID it is multifactorial.
- There is a skewing of the Th1/Th2 response towards Th1 (proinflammatory).
- There are antibodies or cells that react against self-antigens- B cells, T cells, antigen-presenting cells .
- In ITP the antigen is platelet glycoprotein, found on megakaryocytes and platelets.

Pathophysiology

- The autoantibodies in ITP are predominantly of the IgG subclass, and isotype switching from IgM to IgG requires T-cell help.
- Targeted platelet antigens include: GPIIb/IIIa and GPIb/IX(the most frequently involved)
- Less commonly, GPIa/IIa, GPIV and GPVa.
- Opsonized platelets are removed prematurely by the RES via an Fc-

Pathophysiology

- The autoantibodies may impair megakaryocyte growth and development, platelet release and may also induce apoptosis of megakaryocytes.
- However, 40% of patients with chronic ITP have no detectible autoantibodies.
- This emphasises T cell role in ITP pathogenesis: a direct CD8+ T cell-mediated cytotoxicity that induces platelet destruction is implied.



Primary immune thrombocytopenia (ITP)

Presentation of ITP :

- Can occur at any age.
- presentation is usually with sudden onset of petechiae, particularly around the feet, and bleeding (nose, gums, bowel, urinary tract).
- For the majority of patients, ITP is a fairly minor disorder- serious bleeding is not common and clinical sequelae of the disease are generally absent in patients with platelet counts above $30 \times 10^9/L$.
- Diagnosis of ITP is of exclusion.
- Treatment: either steroids or hdIVIg

Thrombotic thrombocytopenic purpura (TTP)

- Thrombotic thrombocytopenic purpura (TTP) is a syndrome characterized by disseminated thrombi in the terminal circulation of several organs, mainly composed of platelets.
- TTP is more frequent in women (two-thirds of cases).
- Congenital (AR) or acquired (autoimmune).

Autoimmune TTP

- Primary (idiopathic) or secondary.
- The most common physiological or pathological conditions associated with autoimmune TTP are: pregnancy, infections, immunomediated diseases, the use of drugs such as ticlopidine and clopidogrel, secondary to metastatic tumours and organ transplantation.

Autoimmune TTP

- In TTP patient plasma contain highly thrombogenic (ultralarge) forms of the multimeric glycoprotein von Willebrand factor (VWF)- ultra-large multimers are normally absent in the plasma.
- ADAMTS13 is also deficient in the plasma of TTP patients contributing to persistence of the vWF ultralarge multimers.

Autoimmune TTP

- ADAMTS13 is a metalloprotease that functions to regulate the size of VWF by disposing of ultra-large multimers secreted from endothelial cells into plasma- thereby preventing heightened platelet aggregation and thrombus formation.

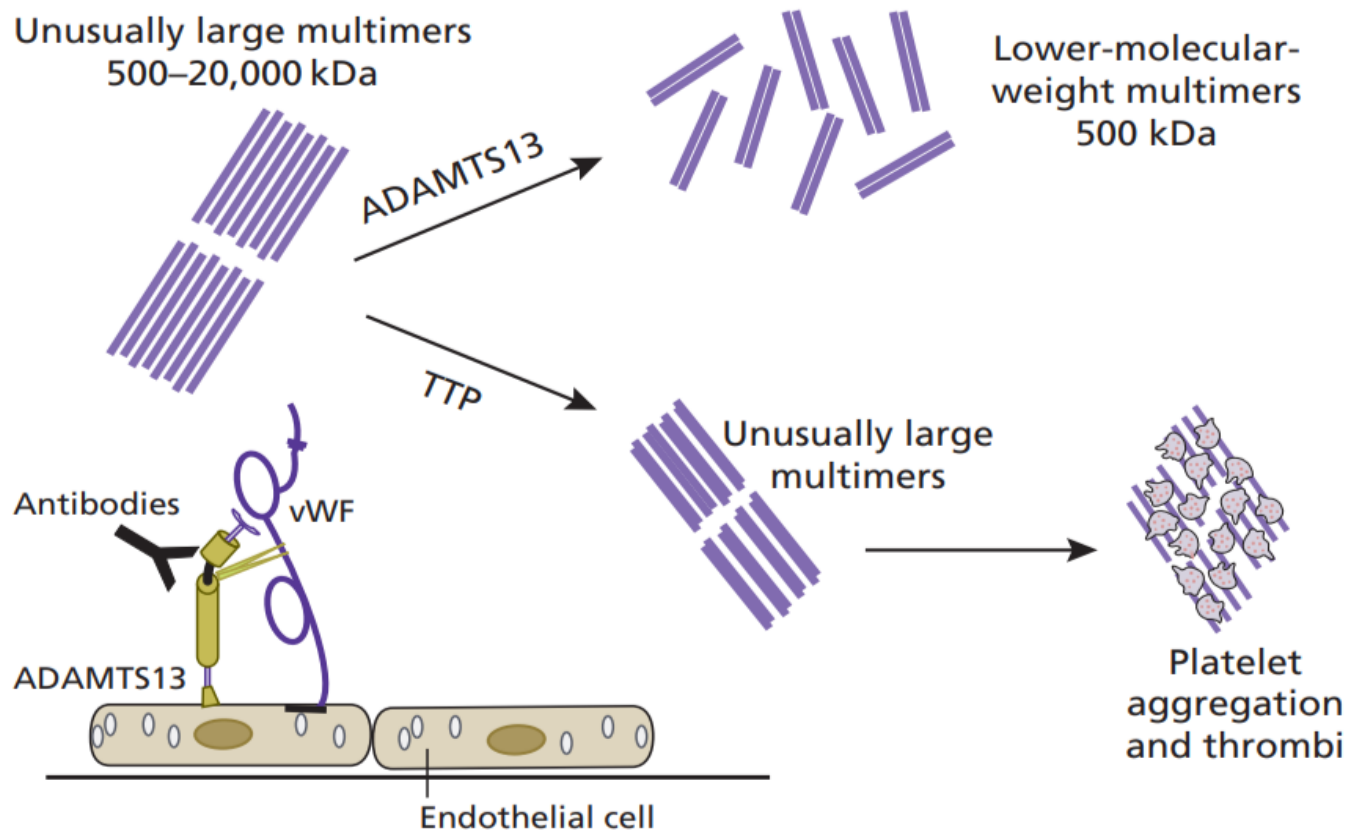
Autoimmune TTP

- Two main mechanisms cause ADAMTS13 deficiency in TTP: mutations in the gene that encodes the protease and the acquired development of anti-ADAMTS13 autoantibodies.
- Acquired TTP is mainly due to autoantibodies to ADAMTS13.

Autoimmune TTP

- Antibodies in autoimmune TTP inhibit the proteolytic activity of ADAMTS13 and/or bind the protease to accelerate its plasma clearance through opsonization and/or other mechanisms.
- Anti-ADAMTS13 are usually of the IgG type- polyclonal IgG4.

Pathophysiology of TTP



Autoimmune TTP

The current mechanistic model of TTP pathogenesis implies that:

- Endothelial cells, activated by varied and often unidentified triggering agents, secrete larger amounts of ultra-large, uncleaved VWF.
- This in the presence of ADAMTS13 deficiency, aggregates platelets directly- especially in conditions of high shear force
- This leads to massive intravascular platelet aggregation, ischaemic organ damage, consumptive thrombocytopenia and mechanical haemolytic anaemia.

Autoimmune TTP

- There is widespread formation of platelet thrombi with abundant intrathrombus VWF in the microcirculation.
- Microthrombi are found in several organs (mainly brain, kidney, myocardium, adrenal gland, digestive tract and pancreas) and lead to multiorgan failure.
- Treatment: plasmapheresis and replacement with fresh frozen plasma $\pm$ immunosuppressive therapy.

Autoimmune neutropenia (AIN)

- Autoimmune neutropenia (AIN) is a rare condition caused by antibodies directed against neutrophil-specific antigens leading to their destruction.
- Primary or secondary.
- Acute or chronic.
- Acute autoimmune neutropenia can be related to drug-induced mechanism or viral infections.
- Chronic autoimmune neutropenia occur in the context of autoimmune diseases, primary immune deficiency syndromes, solid tumours or haematological malignancies- such as large granular lymphocyte leukaemia.

Physiopathology

- The acquired autoimmune neutropenia are characterized by the presence of an antibody, usually IgG, directed against PMN specific antigens.
- Anti-neutrophil antibodies, called Human Neutrophil Alloantigen (HNA) antibodies are directed against neutrophil surface glycoproteins.
- These autoantibodies agglutinate neutrophils, which are then phagocytized.
- Opsonized neutrophils are cleared from the circulation by the spleen and by various tissues containing phagocytic cells.

Physiopathology

- Neutrophil surface antigens identified in primary autoimmune neutropenia are antigens in the HNA system.
- HNA-1 or CD16b (FcGR1) and HNA-2 or CD177 (NB1 glycoprotein) are the major immunogenic molecules of the neutrophil membrane.
- They are both specific for the neutrophil cell.

Physiopathology

- There is no a direct correlation between the amount of autoantibodies and the degree of neutropenia.
- This indicates role of complement activation, which amplifies neutrophils destruction.
- Furthermore, antibodies can be targeted against a progenitor or a mature cell.
- Antibodies can also affect neutrophils function in a qualitative way by causing a defective response to chemotaxis.

Autoimmune neutropenia (AIN)

- All patients will require a bone marrow examination if neutropenia is severe and persistent.
- Screen for associated autoimmune disease (ANA, dsDNA, ENA, TPO, GPC).
- Attempts to detect anti-neutrophil-specific antibodies are hampered by the non-specific binding of immunoglobulins via neutrophil Fc receptors.
- Treatment: antifungals, G-CSF, steroids, hdIVIg.

