HEMATOLOGY

Immunohematology, transfusion medicine and bone marrow transplantation

Institute of Pathological Physiology

First Facultry of Medicine, Charles University in Prague

http://patf.lf1.cuni.cz

Questions and Comments: MUDr. Pavel Klener, Ph.D., *pavel.klener2@lf1.cuni.cz*

Presentation in points

1. Imunohematology
2. AB0 blood group system
3. Rh blood group system
4. Hemolytic disease of the newborn and neonatal alloimmune thrombocytopenia
5. Pre-transfusion examinations
6. Transfusion reactions
7. Transfusion medicine and hemapheresis
8. HLA system
9. Stem cell/ bone marrow transplantation

Origins of immunohematology and transfusion medicine

Imunohematology as a branch of medicine developed hand in hand with the origins of transfusion therapy. It focuses on concepts and questions associated with transfusion therapy, immunisation (as a result of transfusion therapy and pregnancy) and organ transplantation.

In 1665, an English physiologist, Richard Lower, successfully performed the first animal-to-animal blood transfusion that kept ex-sanguinated dogs alive by transfusion of blood from other dogs.

In 1667, Jean Bapiste Denys, transfused blood from the carotid artery of a lamb into the vein of a young man, which at first seemed successful. However, after the third transfusion of lamb's blood the man suffered a reaction and died.

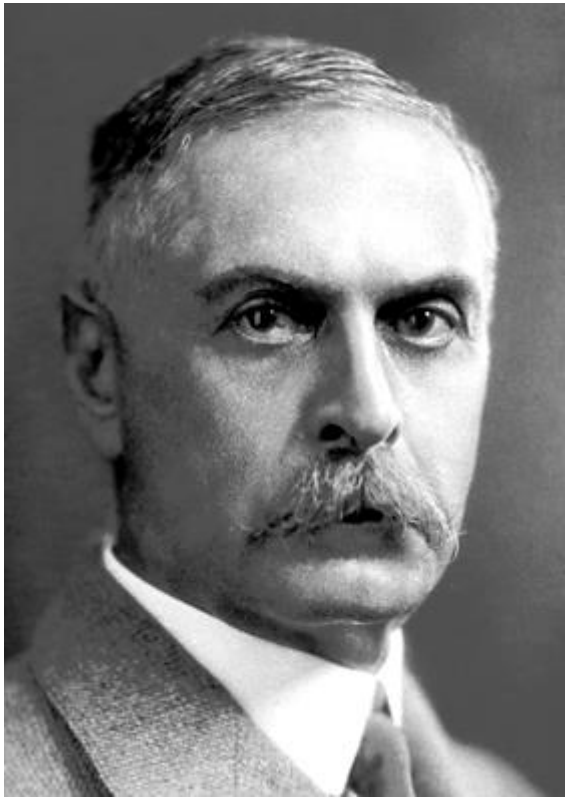
Due to the many disastrous consequences resulting from blood transfusion, transfusions were prohibited from 1667 to 1818- when James Blundell of England successfully transfused human blood to women suffering from hemorrhage at childbirth.

Revolution in 1900

In 1900 Karl Landsteiner discovered the AB0 blood groups.

This landmark event initiated the era of scientific – based transfusion therapy and was the foundation of immunohematology as a science.

In 1930 Karl Landsteiner was awarded a Nobel Price for Physiology and Medicine.

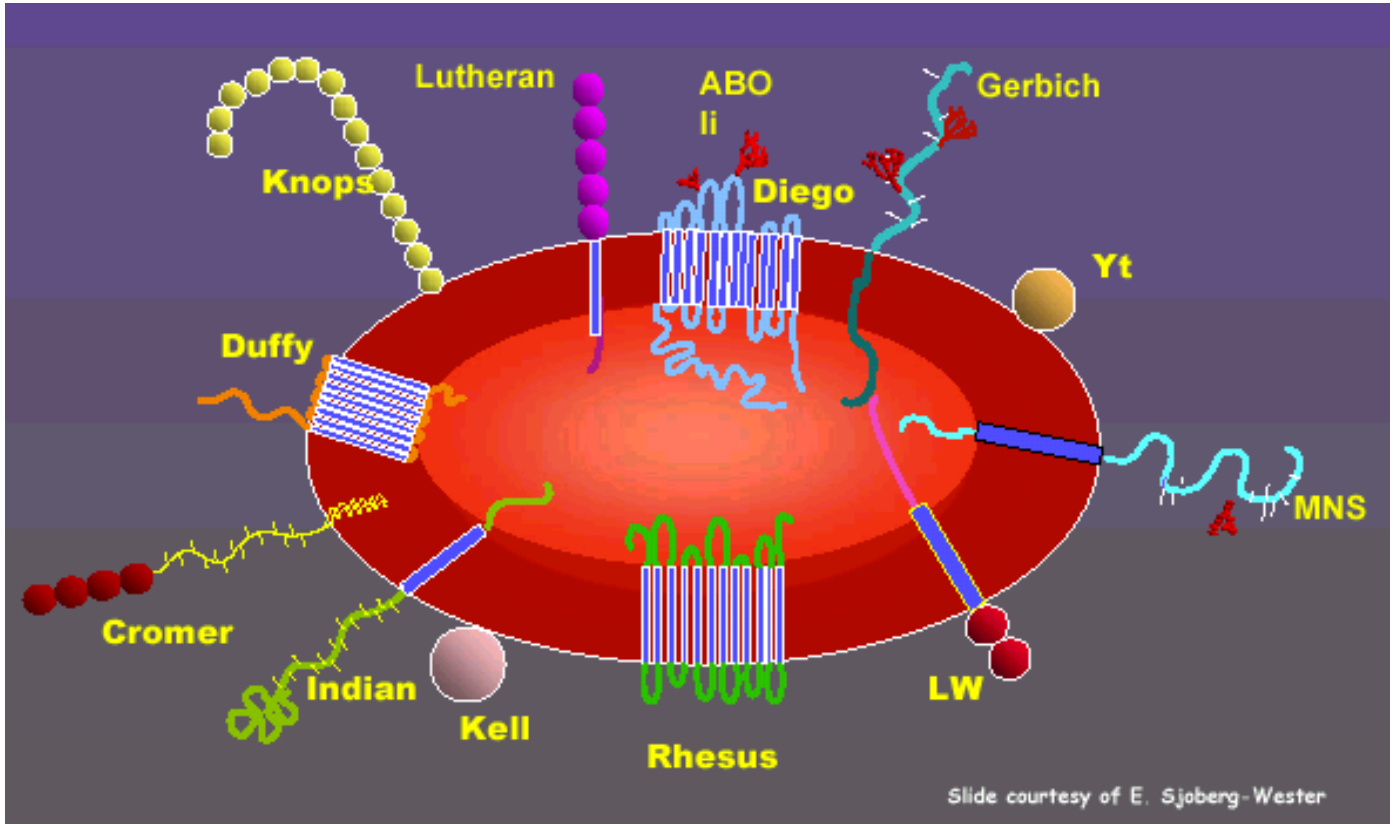


Blood group antigen systems

Up to the present more than >30 blood group systems with > 250 antigens.

Blood group systems can be divided into two large groups:

1. Protein antigens
2. Carbohydrate antigens



Functional characteristics of blood group antigens

Molecular class	*Gene symbol*	*Symbol ISBT*	*Chromosome*	*Protein or lipid*[§]	*Size (kDa)*[°]	*Copies per RBC*	*Biological function*
Transporter or channel	DI	010	17q21	Band 3 (CD233)	90	10^6	Anion exchanger [AE1]
	CO	015	7p14	AQP-1/CHIP28	28**	$2x10^5$	Water channel
	JK	009	18q11-q12	Kidd	50	$15x10^3$	Urea Transporter [hUT-B1]
	RH	004	1p34-p36	Rh (CD240)	30-32	$2x10^5$	RhAG(CD241)* - Amonium Transporter
	XK	019	Xp21.1	Kx	37		Transporter?
Receptor	FY	008	1q22-q23	DARC (CD234)	35-45	$15x10^3$	Receptor *P. vivax* / chemokines / (HIV-1 ?)
	KN	022	1q32	CR1 (CD35)	170-280	10^3	Receptor *P. falciparum* / C3b, C4b
	MNS	002	4q28-q31	GPA/B (CD235A/CD235B)	36/20	$10^6/3x10^5$	Receptor *P. falciparum (EBA-175)*/ bacteria / viruses
	CROM	021	1q32	DAF (CD55)	70	$6\text{-}15x10^3$	Receptor *E. coli* / Enterovirus
	P	003	22q11-ter	Globoside		$10x10^6$	Receptor Parvovirus B19
Adhesion	IN	023	11p13	CD44	80	$5\text{-}10x10^3$	Ligands= Hyaluronate, Collagens I and VI, fibronectin, Serglycin, ETA-1
	LW	016	19p13	ICAM-4 (CD242)	42	$3\text{-}5x10^3$	Ligands= integrins $\alpha4\beta2$ (and $\alpha4\beta1$, $\alpha v\beta3$?)
	LU	005	19q12-q13	Lu/B-CAM (CD239)	78-85	$1.5\text{-}4x10^3$	Ligand= Laminin (chain $\alpha5$)
	XG	012	Xp22-p32	XG1(Xg^a)/XG2(CD99)	22-29	150/960	? ligand ?
	OK	024	19p13.2	EMMPRIN (CD147)/Ok^a	54-65		Leukocyte adhésion molecule M6 (ligand = ?)
	JMH	026	15q23-q24	JMH/SEMA7A (CDw108)	75-80		Semaphorin 7A (Cell attachment through RGD sequence ?)
Enzyme	ABO	001	9q34-q34.2	GlycosylTransferase	40-42		3-α-D-GalNAc/Gal-transferases (A/B)
	H	018	19q13	GlycosylTransferase			2-α-L-fucosyltransferase (H= FUT1 / SE= FUT2)
	LE	007	19p13	GlycosylTransferase			3/4-α-L-fucosyltransferase (FUT3)
	YT	011	17q22.1-22.3	Cartwright	160	$3x10^3$	Acetylcholinesterase
	KEL	006	7q32-q36	Kell (CD238)	93	$3\text{-}6x10^3$	Zn-Metalloproteinase
	DO	014	12p13.1-13.2	Dombrock	54-57		ADP-ribosyltransferase ?
Structure+	GE	020	2q14-q21	GPC/D (CD236C/CD36D)	32/23	$2x10^5$	Mechanical/elastic properties of red cell membrane and receptor *P. falciparum (BAEBL)*
Others	CH/RG	017	6p21.3	C4A/CAB fragments			*Complement fractions adsorbed on RBCs*
	SC	013	1p32-p34	Scianna	*60*		*unknown*
	RAPH	025	11p15	MER2	*40#*	*70-500#*	*unknown*

Natural antibodies, immunization, and allo-antibodies

Natural antibodies are red cell antibodies in the serum of an individual that are not provoked by previous red cell sensitization. They develop in all genetically predisposed individuals (e.g. Anti-A IgM antibody in an individual with blood group B).

Natural antibodies are probably formed in response to antigen determinants present on bacterial strains of GIT, which mimick blood group antignes (AB0, P, Le etc.).

Immunization= formation of antibodies (=**allo-antibodies**) against (**foreign**) antigens present on donor red blood cells, lymphocytes, or platelets that are not present on recipient blood cells.

Most common causes of immunization:

1. **Transfusion** of blood and blood derivatives
2. **Pregnancy**

Different clinical relevance of antibodies in transfusion medicine:

ABO→ K (Kell system) → E (Rh systém)→ c (Rh system)→ Fya (Duffy system)→ C (Rh system)→ Jka → S (MNS system)→ Jkb

Antibodies

	IgG1	IgG2	IgG3	IgG4
%	65	25	6	4
Complement fixation	+4	+2	+4	+/-
Biologic half-life (days)	22	22	8	22
Passage through placenta	Yes	Yes	Yes	Yes
	Anti-Rh	Immune anti-AB0	Anti-Rh	Immune anti-AB0

Carbohydrate blood group antigens: AB0 system

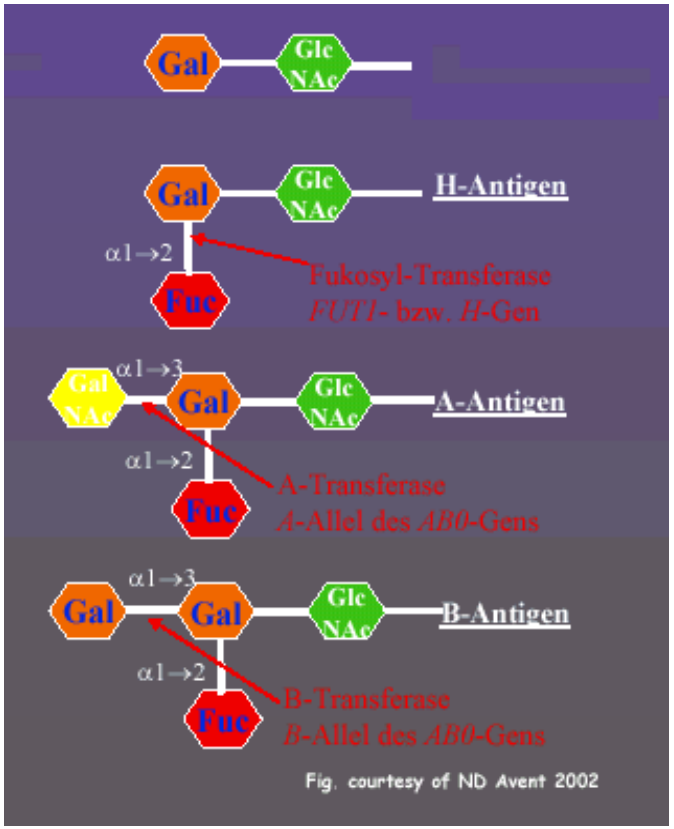
AB0 system

AB0 represents the most important blood group systém.

AB0 incompatibility was the most important cause of disasterous results of blood transfusion attempts before Landsteiner.

- Gen (DNA)
- RNA
- Glykosyltransferase
- Antigen

Addition of the fucose on the precursor sugar frame depends on presence of **H-transferase**.



AB0 system

ABH antigens are expressed on glycoproteins and glykolipids of most tissues = tissue antigens → they play important role in solid organ transplantation

ABH antigens can be secreted into plasma and body fluids (secretory phenotype, 80% population)

	Gene	Fucosyl-transferase	Phenotype
1.	H-transferase (FUT1)	→ Fukosyl-transferase 1	→ H antigen on surface of RBC
2.	Se-transferase (FUT2)	→ Fukosyl-transferase 2	→ secretion of antigens
3.	h/h + se/se	→ no FUT1/FUT2	→ Bombay phenotype (O_h)
4.	h/h	→ no FUT1	→ Para-Bombay phenotype

AB0 system

Branching of the precursor sugar frame is then carried out by <u>A- and B-transferases</u>

	Gene	Glycosyl-transferase (sugar)	Antigens	Blood group
1.	A	→ A- transferase (D-galactosamine)	= A + H antigens	= group A
2.	B	→ B- transferase (D-galactose)	= B + H antigens	= group B
3.	A+B	→ A + B- transferase (both sugars)	= A + B + H antigens	= group AB
4.	0 (=amorphic allele)	→ no enzyme (no sugar)	= only H antigen	= group 0

Bombay a Para-Bombay fenotyp

Approx. 20% population lack a functional gene for FUT2 → non-secretory phenotype

ABH are expressed on glycoproteins and glykolipids of most tissues = tissue antigens → they play important role in solid organ transplantation

Bombay phenotype (hh)= O_h= absence of FUT1 and FUT2 → no A/B/H antigens

Para-Bombay phenotype = absence of FUT1 → no or very weak A/B/H antigens adsorbed from plasma

A Two-Locus Model for H and Se Phenotypes[a]

	Phenotype	Genotype
Secretor	H(A&B) on red cells	*HH* or *Hh*
	H(A&B) in secretions	*Sese* or *SeSe*
Non-Secretor	H(A&B) on red cells	*HH* or *Hh*
	H(A&B) absent from secretions	*sese*
Para-Bombay	Weak or absent H(A&B) on red cells (antigens adsorbed from plasma)	*hh*
	H(A&B) in secretions	*Sese* or *SeSe*
Bombay (O_h)	H(A&B) absent from red cells	*hh*
	H(A&B) absent from secretions	*sese*

Frequency of AB0

	A (%)	B (%)	AB (%)	0 (%)
Europe	40	11	4	45
Asia	28	27	5	40
Africa	26	21	4	49

Probability of AB0 incompatible blood transfusion before Landsteiner was **35.59%**.

AB0 system and hemolytic disease of the newborn (HDN)

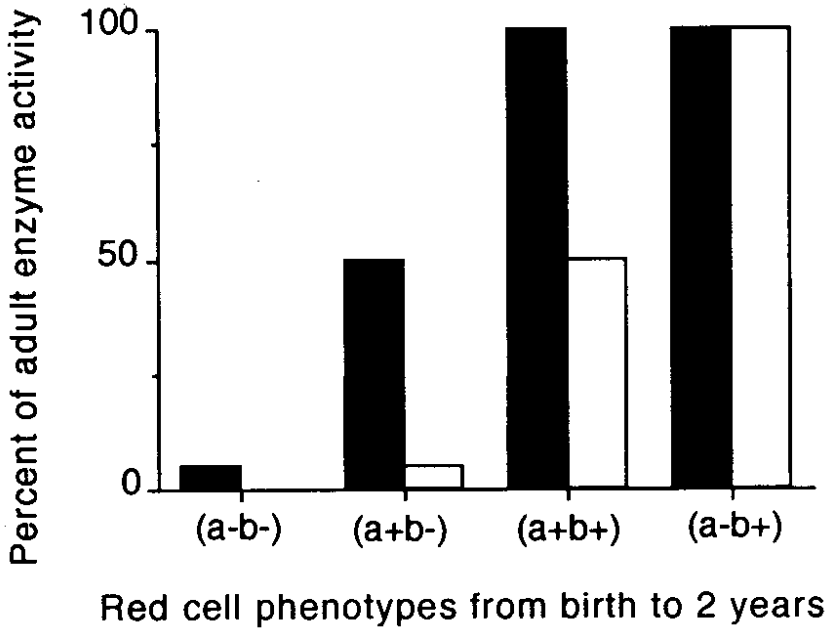
AB0-incompatible transfusion → life-threatening complication due to **intra**vascular hemolysis (polyvalent IgM antibodies)

AB0-incompatible HDN → usually only mild consequencies

← Only IgG gets through the placenta (not IgM pentamers)

-ABH antigens are not fully developed on placental erythrocytes

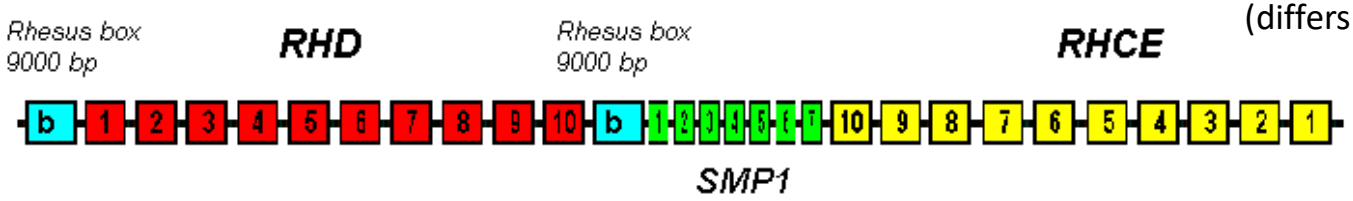
-tissue ABH antigens absorbe (and titer) anti-ABH antibodies



Protein antigen blood group systems: Rh system

Rh system

RhD gene= 10 exons = 30kD
RhCE gene= 10 exons = 30kD
(differs from RhD by only 35 AA)



Rh system is the most polymorphic blood group system: >50 antigens

Serologically >70 phenotypes

Genetically > 200 alleles

Major antigens: **D**, C, c, E, e… and many many more….(most frequently HFA or LFA (high-frequency antigens or low-frequency antigens) expressed only on red blood cells

A person is grouped as Rhesus (Rh) positive or negative based on the presence or absence of antigen D.

The ‘d’ gene is not expressed and **there is no ’d’ antigen, it only implies absence of ’D’**.

Individuals who lack any of the Rh systém antigens may be stimulated to produce the corresponding antibodies (anti-D, anti-C, anti-c, anti-E, anti-e) by transfusion or pregnancy.

Rh system

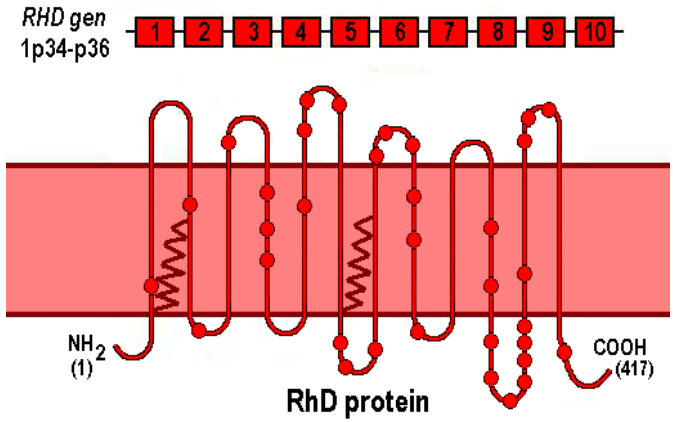
D-antigen= **<u>collection</u>** of conformation-specific antigens that arise along the whole RhD protein

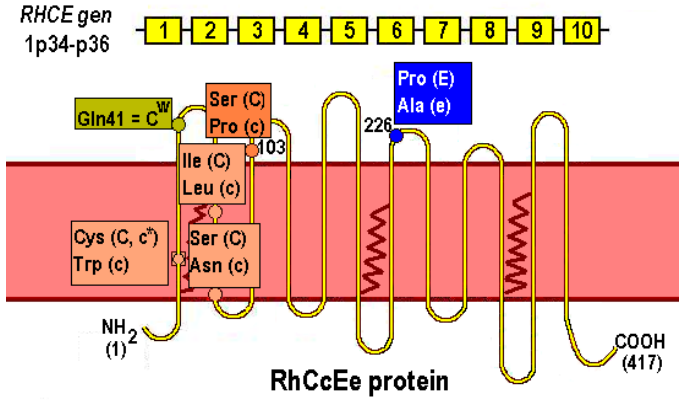
C/c antigens differ from each other by a single AA (P103S)

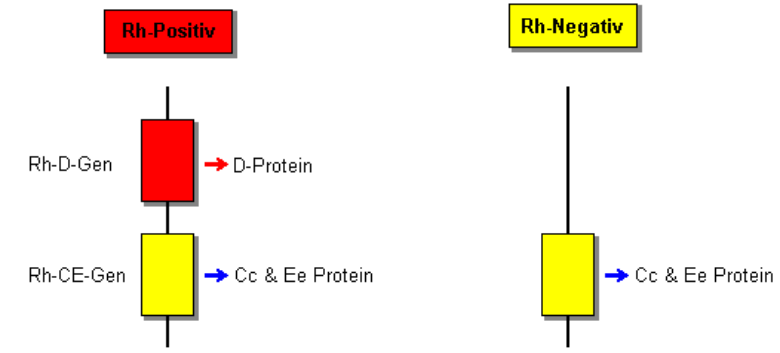
E/e antigens differ from each other by a single AA (A226P)

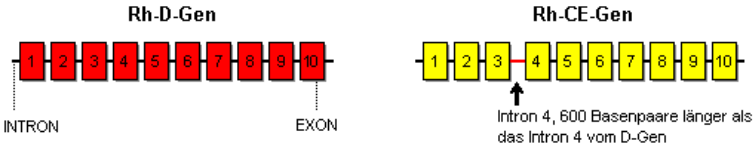
Rh-<u>negative</u>= D-negative (15% Caucassians, 5% Blacks, <0.1% Asiatics)

Rh-<u>null</u>= as a consequence of a mutation of RhAG (Rh antigens are expressed only in complex with RhAG)









Rh system

Rh system is the most common cause of (allo)-immunisation → the second most common cause of transfusion reactions

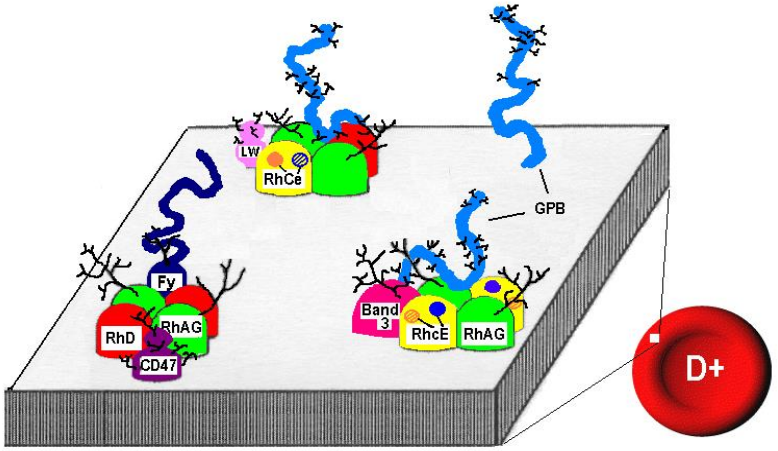
Rh system is the most common target of auto-antibodies (in WAIHA)

Rh system is the most common cause of serious HDN

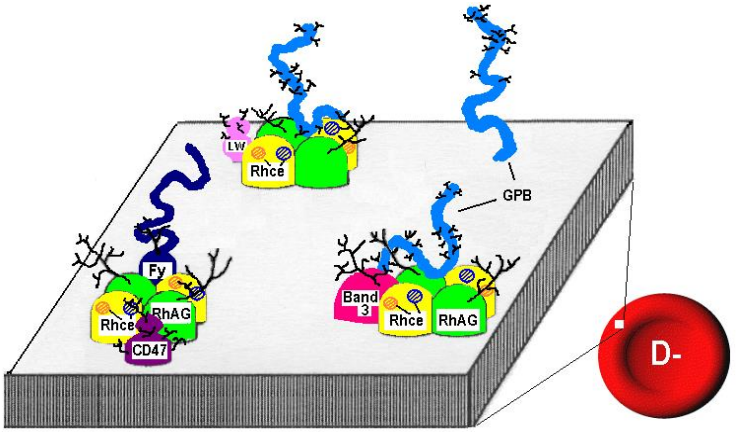
The Rh antigens can be demonstrated on fetal red cells as early as 38 days after conception, and are **well developed at birth**.

Frequency of Rh haplotypes

Haplotype	Europe (%)	Africa (%)	Asia (%)
DCe	41	6	73
dce	39	20	2
DcE	14	12	19
Dce	3	59	3
dcE	<1	<1	<1
dCe	<1	3	2
DCE	<1	<1	<1
dCE	<1	<1	<1



RhD+ (D+ C+ c+ E+ e+) *RHD-RHCe/RHD-RHcE*



RhD- (D- C- c+ E- e+) *RHce/RHce*

Frequency of Rh phenotypes

Phenotype	Frequency (%)	Anti-Rh antibodies
DCe-dce	34	anti-E
DCe-DCe	17	anti-E, anti-c
dce-dce (=D-negative)	**15%**	anti-D, anti-C, anti-E
DCe-DcE	12	netvoří protilátky
DcE-dce	12	anti-C
DcE-DcE	2	anti-C, anti-E

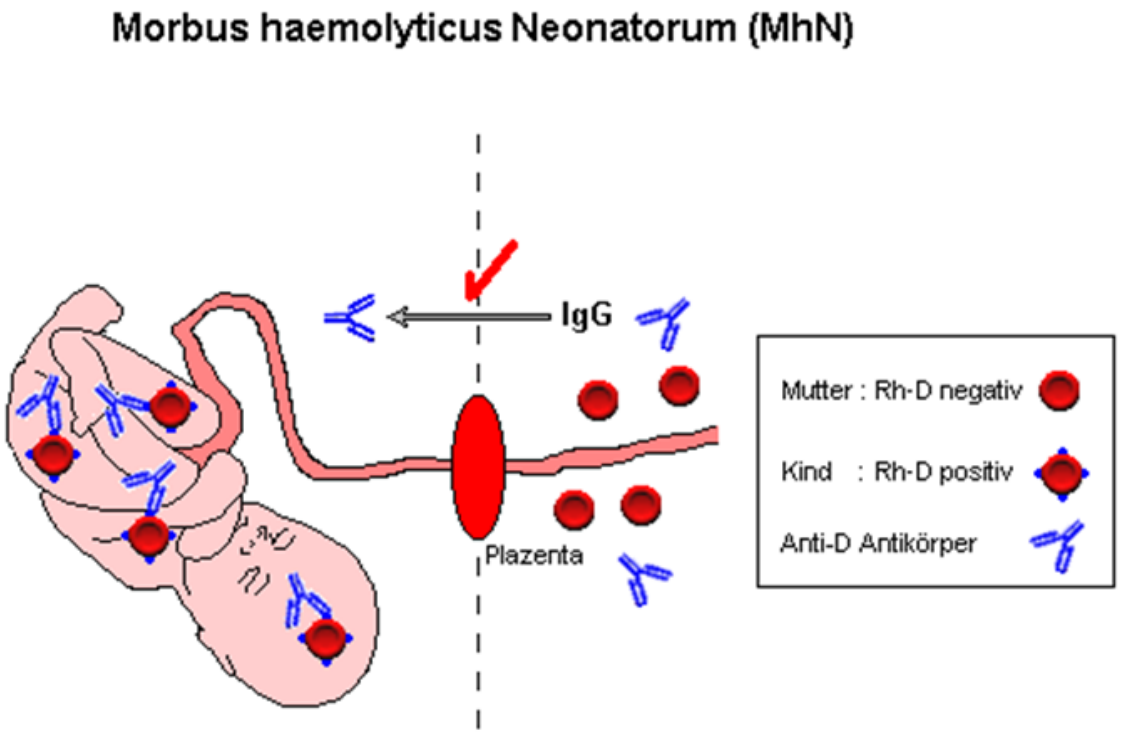
Hemolytic disease of the newborn (HDN)

HDN

Presence of an antigen on fetal red blood cells that is absent on maternal red blood cells

→ Immune hemolysis of fetal red blood cells by maternal antibodies.

HDN rarely manifests during the first pregnancy (predominantly in allo-immunized women).



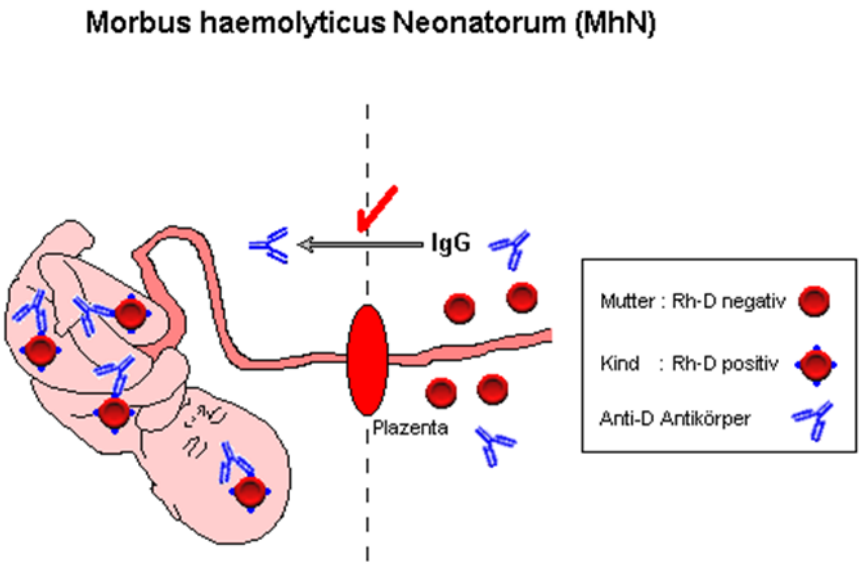
HDN

Survival of normal fetal erythrocytes = 45-70 days.

Antibodies → survival < 45 days.

Immunization → production of maternal IgG antibodies that pass from the serum of the mother through the placenta into the fetal circulation and bind to fetal red blood cells = red blood cell sensitization→ premature destruction of sensitized fetal red blood cells in the spleen and liver → anemia → hepatosplenomegaly due to increased destruction of red blood cells and due to stimulation of extramedullar hematopoiesis → portal hypertension → cardiac insufficiency→ generalized oedema → hydrops foetalis

Hemolysis → accumulation of bilirubin (=product of extravascular hemolysis) with ensuing damage of fetal brain structures.

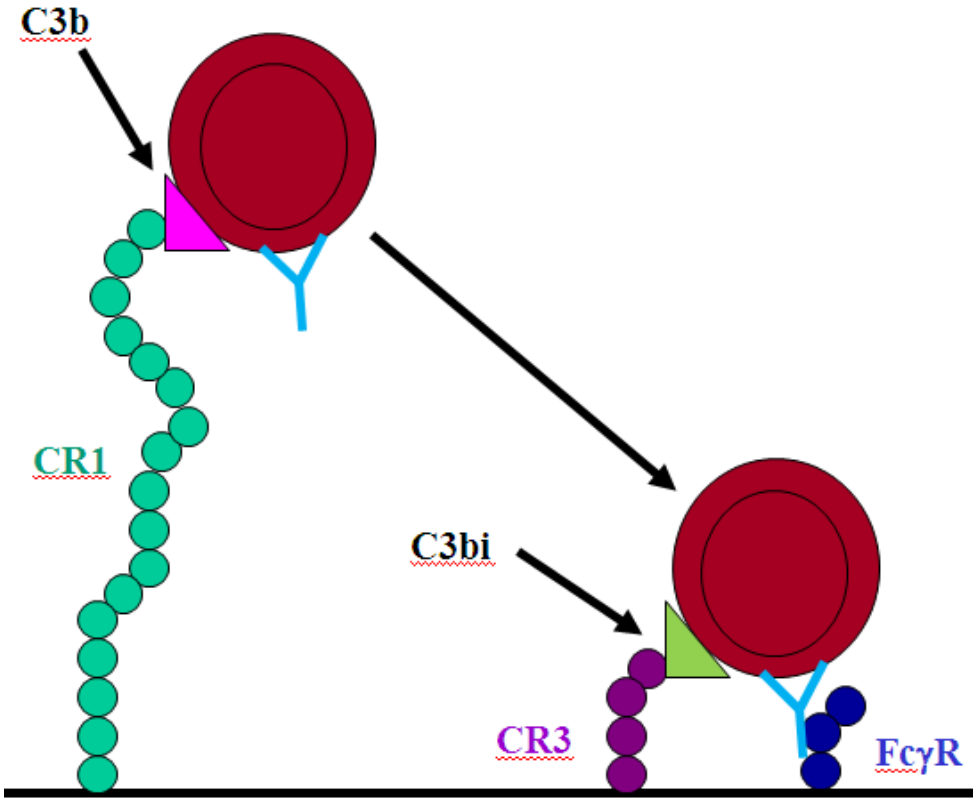


HDN- antibodies

Antibodies against **Rh system** (D, c, E).

As low as 100uL of fetal Rh+ red blood cells elicit primary immune reaction (immunization).
As low as 30uL fetal Rh+ red blood cells elicit secondary immune reaction.

Clinically relevant antibodies= IgG1a IgG3 that bind to Fc-gamma receptors and on complement receptors (CRs) on splenic and liver macrophages.



HDN and AB0 system

Only mothers that type „0“ develop during pregnancy allo-antibodies IgG anti-A and anti-B.

→ Only rarely leads to clinically relevant HDN, but can manifest already during the first pregnancy.

Antibodies agains **Kell**, **Kidd**, **Duffy**, **S** blood group systems can result in clinically relevant HDN.

Examination during pregnancy

-AB0 RhD blood group typing

-examination of allo-antibodies twice during pregnancy: 12th week, and 28th week.

In RhD-negative (D-negative) women, weak D must be excluded.

RhD status of the father must be examined.

Assessment of the titer of the clinically-relevant antibodies in 16 week of pregnancy → re-assessment in 2-week intervals.

Hyperimmune anti-D serum (partobullin).

Within 72 hours of delivery in Rh-negative mothers of Rh-positive newborns.

300ug anti-D will inhibit immunization induced by 30mL of fetal red blood cells.

Neonatal alloimmune thrombocytopenia

Alloimmune thrombocytopenia

-antibodies against human platelet antigens (HPA) present on fetal platelets.

-most frequently HPA1b homozygous mothers produce antibodies against HPA1a (=gpIIbIIIa) expressed on fetal platelets → thrombocytopenia already at the end of 20th week of gravidity.

-50% occur during the first pregnancy

Incidence= 1 / 2-3.000 deliveries.

Mortality= 10%.

Pre-transfusion examination

Testing principles: agglutination and hemolysis

The observable reactions resulting from the combination of a red cell antigen with its corresponding antibody are agglutination and/ or hemolysis.

Agglutination is the widely observed phenomenon in blood grouping.

Agglutination: is the clumping of particles with antigens on their surface, such as erythrocytes by antibody molecules that form bridges between the antigenic determinants.

Hemolysis: is the break down or rupture of the red cell membrane by specific antibody (hemolysin) through the activation of complement with the release of hemoglobin, and

the librated hemoglobin can easily be observed staining the supernatant fluid.

Antisera

An antiserum is a purified, diluted and standardized solution containing known antibody, which is used to know the presence or absence of antigen on cells and to phenotype once blood group.

Antiserum is named on the basis of the antibody it contains:

- Anti- A antiserum which contains anti- A antibody

- Anti- B antiserum which contains anti- B antibody

- Anti- AB antiserum, which contain both anti A and B

antibodies.

- Anti –D antiserum which contains anti- D antibody

Agglutination

1. Sensitization/coating

-binding of antibody to surface of RBCs

2. Bridge formation

-linkage of adjacent RBCs that are coated with antibody

RBCs are naturally repelled by the negative charges due to sialic acid at their surfaces

= **“zeta potential”** → RBCs usually don’t get closer than about 25nm apart.

Zeta potential can be reduced during testing proceedures by:

-low ionic strength solutions (LISS) or albumin (fewer ions to surround RBCs)

-water-exclusion (e.g. polyethlyene glycol, polybrene, dextran)

-centrifugation forces

-enzymes (papain, pepsin → cleave out sialic acid)

Coombs tests

Anti-human globulin (AHG)

Types of anti-human globulin (AHG)

1. Polyclonal anti-IgG + monoclonal anti-C3d

2. Anti-IgG

-used for gel and solid-phase platforms exclusively

3. Anti-C3d

-useful for evaluating IgM-related hemolysis and cold agglutinin disease, where antibodies are not usually detectable via anti-IgG

Direct agglutination- Direct Coombs test (DAT)

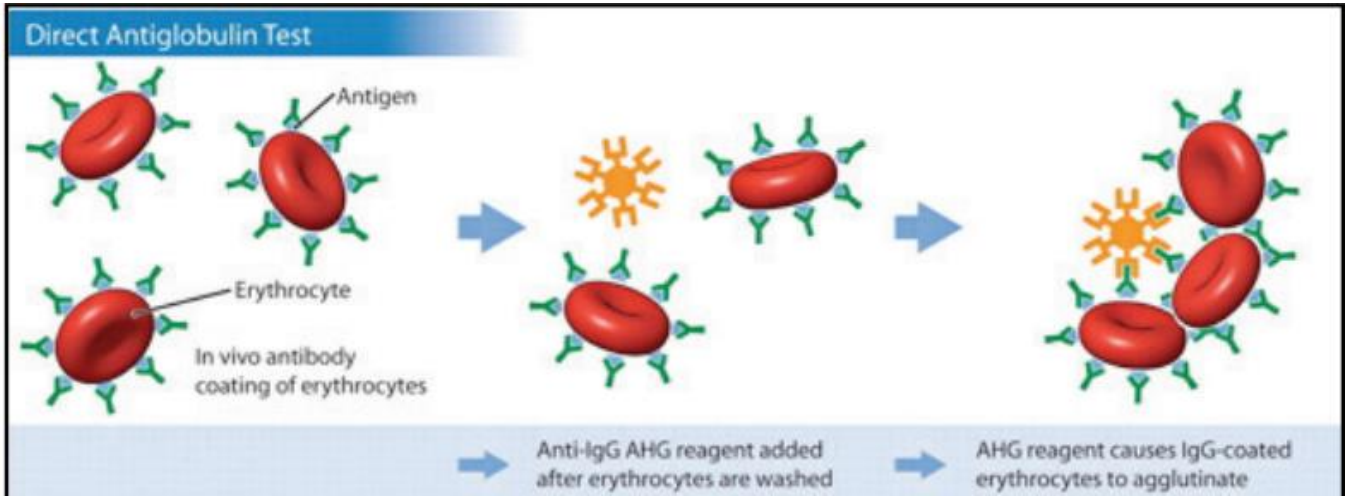
Direct agglutination:

Antibody binds to multiple RBCs and causes agglutination without additional manipulation.

To overcome RBC zeta potential IgM works far much better than IgG (IgM maximum diameter is 30nm compared to 14nm for IgG)

It is used to demonstrate whether red cells have been sensitized (coated) with antibody or complement in vivo, as in case of **HDN**, a**utoimmune haemolytic anemia**, **drug-induced haemolytic anemia**, and **transfusion reactions**.

-at least 300 Ab molecules required for the test to be positive.



Indirect agglutination- indirect Coombs test (IAT)

Antibody binds to, but does not form bridges with, RBCs (IgG).

Test requires additional step to see agglutination= addition of antihuman globulin (AHG).

AHG= antibody against human IgG and/or against C3d component of the complement.

→ detection of antibodies that may cause red blood cell sensitization and subsequent reduced survival of transfused red cells or even cause hemolytic transfusion reactions (most frequently delayed) → **screening of allo-antibodies during pre-transfusion examination.**

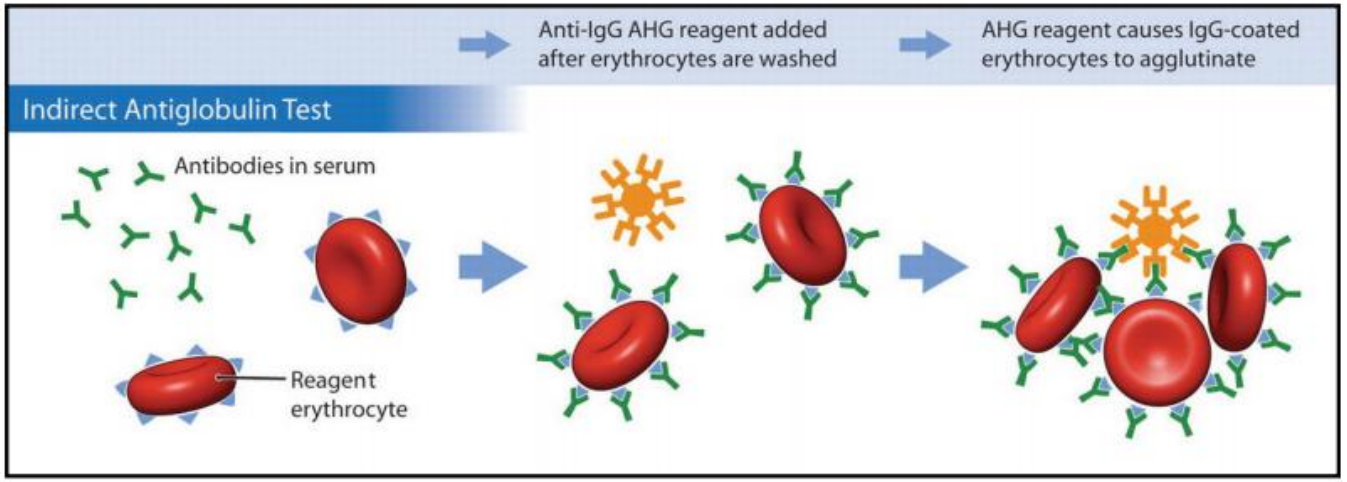
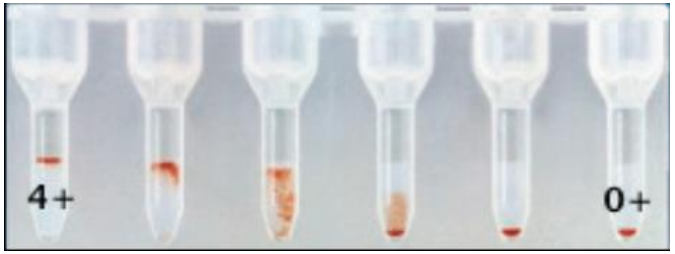


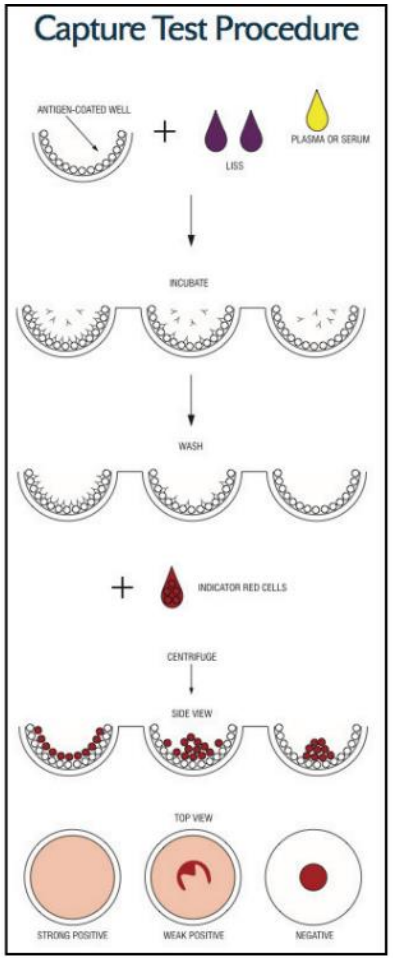
Image credit: Can Med Assoc Journal Jan 2006

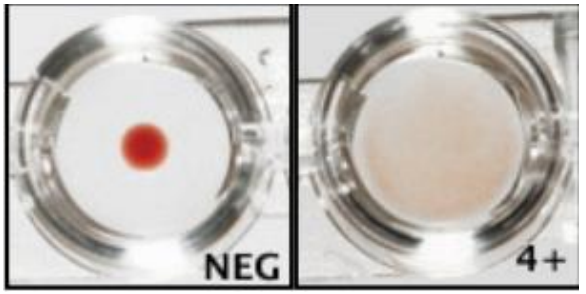
Evaluation of agglutination reactions

Column (gel) agglutination



Solid phase agglutination





Adsorption and elution of alloantibodies

Adsorption= removal of specific antibodies from sample via incubation with antigen-positive RBCs.

Adsorption is used to remove warm or cold autoantibodies (“autoadsorption”) from sample in order to detect underlying alloantibodies.

Adsorption may also be used to remove one or more alloantibodies (“alloadsorption”) from sample in order to detect or confirm the presence of other alloantibodies.

Elution= removal of the antibody from the red blood cell → the resulting eluate is tested for antibody specificity.

-HDN (fetal red blood cells)

-immune hemolytic anemias (=Coombs-positive)

-hemolytic transfusion reactions

Pre-transfusion examination: red blood cells

1. Blood group typing (patient´s red blood cells): AB0 + RhD (patients RBCs + antibodies anti-A, anti-B, anti-D1, anti-D2; diagnostic RBCs + patients serum)

2. Screening for allo-antibodies (patient´s serum)

-examine patient´s serum + 3 diagnostic red blood cells for presence of alloantibodies

3. Choosing compatible donor red blood cells

4. Patient-donor compatibility testing:

-re-confirm AB0 blood group of the patient´s blood

-re-confirm AB0 blood group of the donor blood

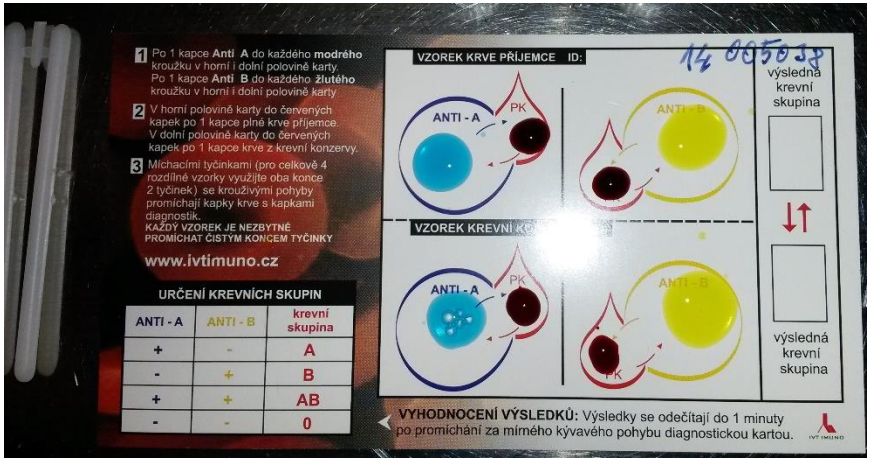
-examine cross-reactivity between the patient´s serum and the donor red blood cells

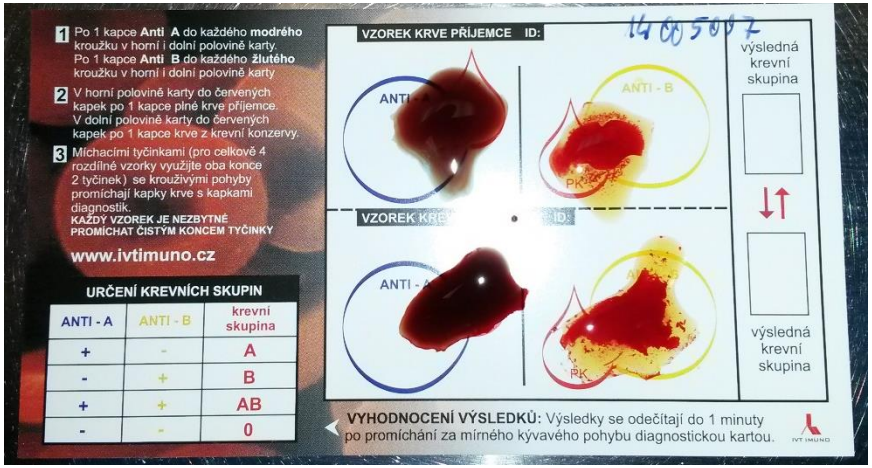
5. Confirmation of blood groups

-bed-site testing

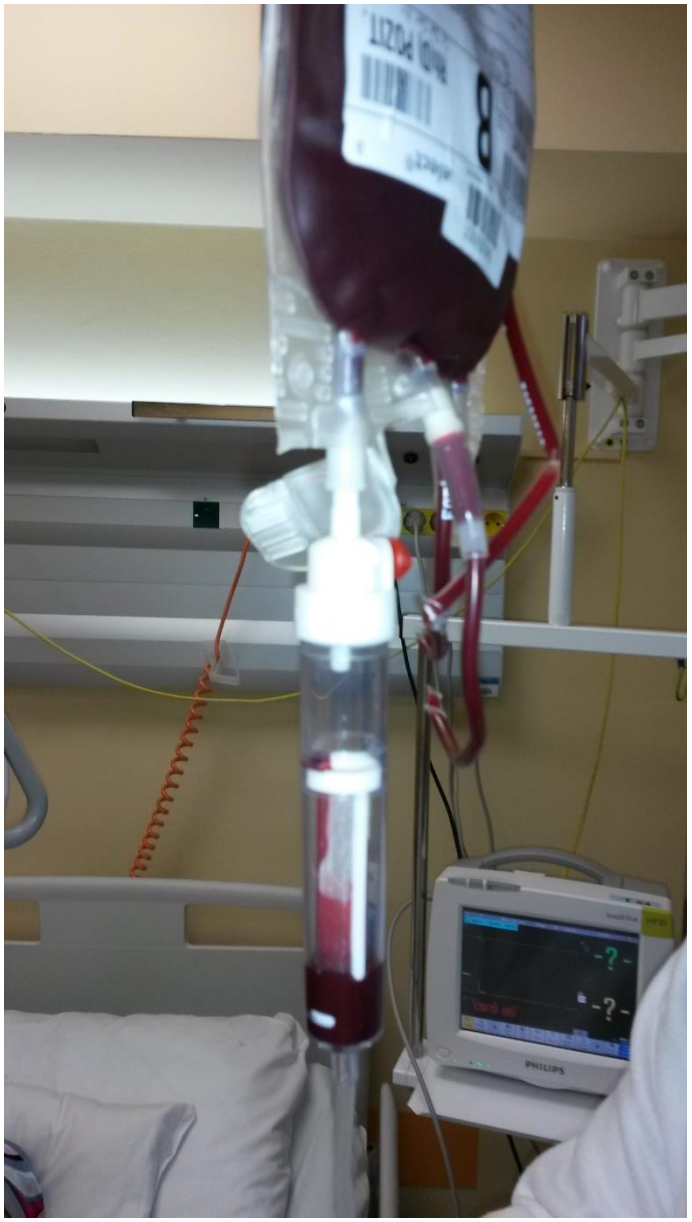
6. Biological testing (transfuse 10mL donor blood → stop and wait for 10 minutes → repeat once more)

Bed-site test: verification of AB0 blood cell group





Podání erymasy



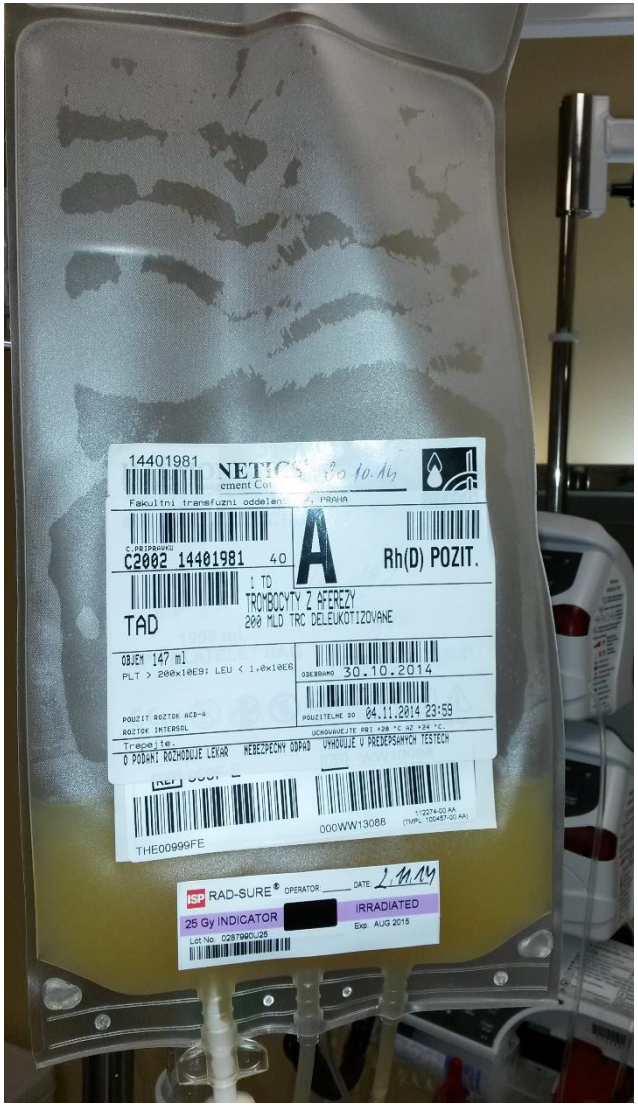
Pre-transfusion examination: platelets, plasma

Platelets: AB0 and Rh compatible

-platelets do not express Rh antigens, but residual red blood cells do.

Plasma: AB0 compatible

Podání destiček



Transfusion reactions

Transfusion reactions

A. Hemolytic

B. Non-hemolytic

A. Early

B. Delayed

Hemolytic transfusion reactions

1. **Early** hemolytic transfusion reactions

← Intravascular hemolysis ← IgM natural antibodies (AB0-incompatibility)

2. **Delayed** hemolytic transfusion reactions

← Extravaskular hemolysis ← IgG alloantibodies (anti-Rh)

Intravascular Haemolytic Transfusion Reactions

Life threatening complication of AB0-incompatible blood transfusion.

1. Intravascular **hemolysis** (=lysis of red blood cells)

← due to IgM-induced activation of complement cascade (formation of membrane attacking complex, C1>>>MAC cascade).

Intravascular hemolysis → thrombosis → disseminated intravascular coagulation (DIC)

2. **Acute systemic symptoms**

← due to **production of anaphylatoxins C3a and C5a** during complement activation

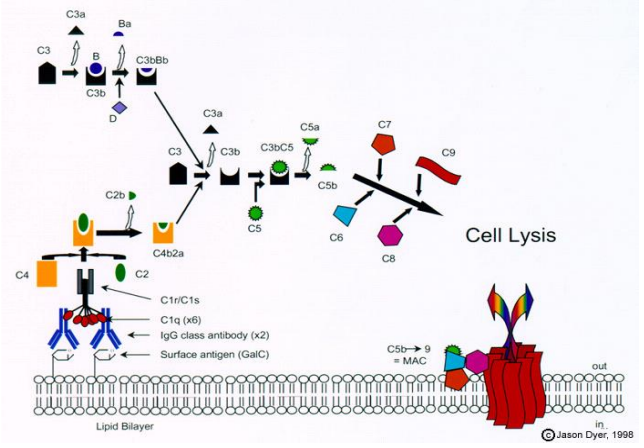
→ smooth muscle contraction

→ histamine and serotonine release from mast cells → enhanced vascular permeability and hypotension → shock

← due to **release of free hemoglobin** into the plasma

→ smooth muscel contraction (free Hb binds nitric oxide)

→ proximal tubule toxicity → acute renal failure



Non-hemolytic transfusion reactions

1. Febrile non-hemolytic transfusion reactions

-alloantibodies in the plasma of the recipient against leucocytes of the donor → release of cytokines from the donor leucocytes.

Prevention of FNHTR= transfusion of leucodepleted blood or platelets

2. Allergic reactions

-most frequently against plasma proteins.

Prevention of alergic reactions=

a. premedication

b. transfusion of washed red blood cells

3. Bacterial contamination

Prevention of bacterial contamination= complex system of multi-level controls during production of blood and blood derivatives

Non-hemolytic transfusion reactions

4. Alloimmunization (most frequently alloantibodies against leucocyte antigens

Prevention of alloimunization= transfusion of leucodepleted blood

5. Transfusion-associated acute lung injury= TRALI

-antibodies in the plasma of the donor against leucocytes of the recepient → release of cytokines from the recipient leucocytes → damage of pulmonary capillaries → capillary-leakage syndrome → pulmonary oedema

6. Transfusion associated graft-versus host disease= TA-GVHD

-a rare, but detrimental complication of blood transfusion in the immunocompromized recipient

Prevention of TA-GVHD= transfusion of irradiated blood and blood derivatives (platelets, plasma)

7. Iron overload

-1mL blood contains approx. 0.5mg iron.

Transfusion medicine

Red blood cell transfusion

1 TU (transfusion unit) of red blood cells is prepared from 450mL whole blood + 63mL CPDA-1.

→ 35 days storage

Content of leukocytes=

Prevention of nonhemolytic febrile reactions < 5x10exp8 leucocytes

Prevention of allo-immunization < 5x10exp6 leucocytes

AB0/Rh compatilibity required. AB0-Rh incompatibility is absolute contraindication for administration of red blood cells.

0 donor= universal donor.

AB recipient= universal recipient.

Platelet transfusion

1 TU whole blood → soft spin → platelet-rich plasma → hard spin → 1 TU plasma + 1 platelet concentrate

1 pletelet concentrate= **5x10exp10 platelets in 50mL plasma**

One adult platelet dose:

=4-6 platelet concentrates pooled together → **2-3x10exp11 platelets**

=**platelets from apheresis → 3x10exp11 platelets** (and only 1x10exp6 leucocytes)

→ 5 days storage under continuous gentel agitation

Platelet refractoriness is most frequently a result of HLA alloimmunization.

Platelets, however, appear to be rather poor immunogens → HLA alloimmunization to platelets is triggered by alloimmunization to contaminating leucocytes.

→ Importance of leukodepleted transfusions.

AB0/Rh- compatibility required, but AB0 incompatibility is not absolute contraindication for platelet administration.

Plasma derivatives

1 TU FFP (fresh frozen plasma)- frozen do < -18 grades Celsius within 6 hours of donation.

-frozen plasma is screened for alloantibodies at the time of collection.

-tests for serologic compatibility are not performed before administration.

-AB0 compatibility required (Rh-compatibility not required)

-AB plasma= universal donor plasma

Hemapheresis

Hemapheresis

=removal of cell or particles from blood by standard centrifugal aphersis equipment

Removal of most blood constituents follows a logarithmic curve → removal of 1.5-2 volumes will reduce an intravascular substance by approx. 80%.

A. Therapeutic <u>cytapheresis</u>:

1. Erythrocytapheresis

-sickle-cell anemia, hemochromatosis, polycytemia

2. Leukapheresis

-leukemias (espec. myeloid leukemias with hyperleukocytosis → depletion)

-peripheral blood progenitor cell (=graft) collection before stem cell therapy

3. Plateletpheresis

-thrombocytemia (depletion)

Hemapheresis

B. Therapeutic plasmapheresis

-TTP (thrombotic thrombocytopenic purpura)

-hyperviscosity

-myastenia gravis

-Guillain-Barré

-cryoglobulinemia

-Goodpasture syndrome

-posttransfusion purpura

HLA system

Human leukocyte antigen (HLA) system

Major histocompatibility complex (MHC) in humans= HLA system (chromosome 6p21).

1. Class I HLA molecules= **monomers** with non-covalently bound beta-2-microglobulin.

-HLA-A/B/C= 3 genes= 6 alleles= **6 different HLA-A/B/C molecules**.

-expressed by all nucleated cells.

→Continuous presentation of cytosolic antigens (in complex with class I HLA molecules)

→ recognition by CD4+ helper T-cells.

2. Class II HLA molecules= dimers (alpha and beta chains that form homo- or hetero-dimers)

-HLA-DR/DQ/DP= up to 20 different HLA-DR/DQ/DP molecules

-expressed by antigen-presenting cells (monocytes, macrophages).

→ Presentation of exo-antigens (e.g. bacterial peptides).

→ Recognition by CD8+ cytotoxic T-cells.

Class II HLA molecules

a. HLA-DR

-HLA-DRA= 1 gene= 2 HLA-DRA alleles

-HLA-DRB= 4 genes (**HLA-DRB1**, DRB3, DRB4, DRB5), but only 2-3 genes are functional in any individual= 6 HLA-DRB alleles

→ 2x6= **8-12 different HLA-DR molecules** (homo- and hetero-dimers)

b. HLA-DQ

-HLA-DQA= 1 gene= 2 alleles

-HLA-DQB= 1 gene= 2 alleles

→2x2= **4 different HLA-DQ molecules** (homo- and hetero-dimers)

c. HLA-DP

-HLA-DPA= 1 gene= 2 HLA-DPA alleles

-HLA-DPB= 1 gene= 2 HLA-DPB alleles

→ 2x2= **4 different HLA-DP molecules** (homo- and hetero-dimers)

HLA system

HLA= the most polymorphic genes with hundreds of alleles:

HLA-B gene → over 1200 alleles

HLA-A → over 800 alleles

HLA-C → over 500 alleles

HLA-DRB1 → over 600 alleles

HLA-DQB1 → over 100 alleles

HLA-DPB1 → over 130 alleles

HLA-DRA1 → 3 alleles

HLA-DQA1 → 34 alleles

HLA-DPA1 → 27 alleles

Clinical HLA testing

1. Serological typing of HLA antigens
2. Genetic analysis (sequencing) of HLA genes

HLA system is inherited as a haplotype in a Mendellian fashion.

-one haplotype inherited from each parent

Search in bone marrow registries all over the world.

Optimal graft donor= HLA-identical.

HLA-A/B/C and HLA-DR-identical (8/8), or event HLA-DQ-identical (10/10).

Stem-cell transplantation (SCT)

Stem-cell transplantation

Stem-cell transplantation= bone marrow transplantation.

= transfer of collected hematopoietic stem and progenitor cells from donor to recipient.

Autologous stem cell transplantation (ASCT):

-donor= recipient

Allogeneic stem cell transplantation (Allo-SCT):

-donor either related (usually a sibling) or unrelater (found in bone marrow register)

= two **fundamentally different** approaches associated with distinctly different morbidity, mortality, and cost.

Stem-cell transplantation

1. Collection of stem and progenitor cells.

-freezing (in case of auto-ASCT)

2. Conditioning.

-high-dose therapy

→ eradication of residual malignant cells

→ immunosuppression

3. Stem cell transfer.

-infusion of collected cells into a large vein via central catheter with a filter.

4. Supportive care.

Aims of auto-SCT

1. Reconstitution of hematopoiesis after high-dose (myeloablative) therapy.

-mature lymphoproliferative neoplasms (multiple myeloma, amyloidosis, aggressive lymphomas).

2. Immunosuppression and re-induction of immune tolerance by myeloblative therapy and reconstitution of lymphopoiesis.

-autoimmune diseases: sclerosis multiplex, scleroderma

Advantages and disadvantages of auto-SCT

Advantages:

1. No graft-vs-host disease
2. Transplantation possible up to 70 years (depending on the „biological“ age)
3. Low morbidity
4. Mortality <2% in specialized centers
5. No need for immunosuppression for graft engrafment
6. No risk of immmunological graft rejection

Disadvantages:

1. No graft-vs-leukemia/lymphoma effect
2. Higher risk of relapse
3. Risk of graft contamination with malignant cells

Aims of allo-SCT

1. Graft-vs-leukemia effect

-acute leukemias

2. Graft-vs-lymphoma/myeloma effect

-salvage therapy of lymphoproliferative disorders (T-NHL, B-NHL, myeloma)

3. Substitution of:

-a defective hematopoiesis (e.g. bone marrow failure syndromes (aplastic anemias), paroxysmal nocturnal hemoglobinuria, sickle-cell anemia, thalassemias)

-a defective immune system (e.g. severe combined immunodeficiency (SCID), Wiscott-Aldrich syndrome (WAS))

-a mutated enzyme (enzymopathies, e.g. mucopolysaccharidoses)

-enzymopathies

Advantages and disadvantages of allo-SCT

Advantages:

1. Graft-vs-leukemia/lymphoma effect
2. Lower risk of relaps
3. No risk of graft contamination with malignant cells

Disadvantages:

1. Risk of graft rejection
2. Risk of graft-vs-host disease
3. Long-term immunosuppression required
4. High morbilidy due to prolonged cytopenias and immunosuppression
5. Long-term mortality up to 20% depending on the disease type, age, performance status and type of graft
6. Risk of CMV re-activation
7. Transplantation up to 60 years

Graft donor

1. HLA-identical sibling

2. HLA-identical or HLA-compatible unrelated donor

3. Haploidentical donor

Graft

Sourace of bone marrow stem and progenitor cells (CD34+):

1. Bone marrow:

-3-4x10exp8 nucleated cells / kg= 10-15mL marrow blood / kg of recipient weight

2. Peripheral blood progenitor cells (PBPC)

-5x10exp6 CD34+ cells / kg of recipient weight

-stimulation of the donor with G-CSF growth factor and collection of PBPC on apheretic machines

3. Cord blood

-2x10exp7 nucleated cells / kg = 10exp5 CD34+ cells / kg (higher percentage of stem cells than in the adult bone marrow blood).

All types of graft are transfused via central venous catethers into large veins (using a filter).

Conditioning

Auto-SCT:

High-dose therapy → anti-tumor effect

Immunosuppression → re-set of immunological tolerance

Allo-SCT:

1. Myeloablative conditioning → anti-tumor effect + immunuosuppression
2. Non-myeloablative (=reduced-intensity conditioning (RIC)) → immunosuppression

Early complications of transplantation

1. Toxicity (mucositis, cystitis, pneumonitis, renal toxicity, myocardial necrosis)

2. Infection (including opportunistic infections, CMV-reactivation)

3. Immunological (hemolysis, graft rejection, graft-vs-host disease, secondary TTP)

Acute GVHD= until 100 days after bone marrow transplantation.

-grading 1-4 depending on the extent of the organ system involvement:

1. **Skin** (percentage of body suface area → generalized bullous erythrodermia)
2. **Liver** (elevation of bilirubin → >255umol/L)
3. **GIT** (diarrhea → ileus)

GVHD diagnosis= biopsy

GVHD therapy= immunosuppression

Chronic GVHD= after 100 days → affects mainly the skin and liver

Late complications of transplantation

1. Sterility
2. Aseptic necrosis of femur
3. Cataracta
4. Alopecia
5. Renal insufficiency
6. Hypertension
7. Loss-off-postvaccination immunity

Fin