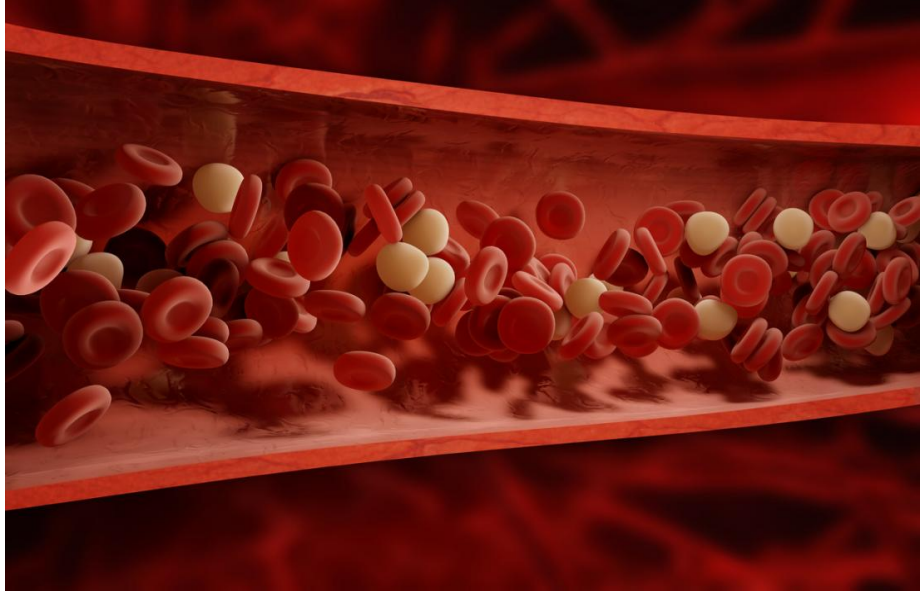
Immunological aspects of transfusion, in vitro antigen-antibody reactions, basics of blood group serology

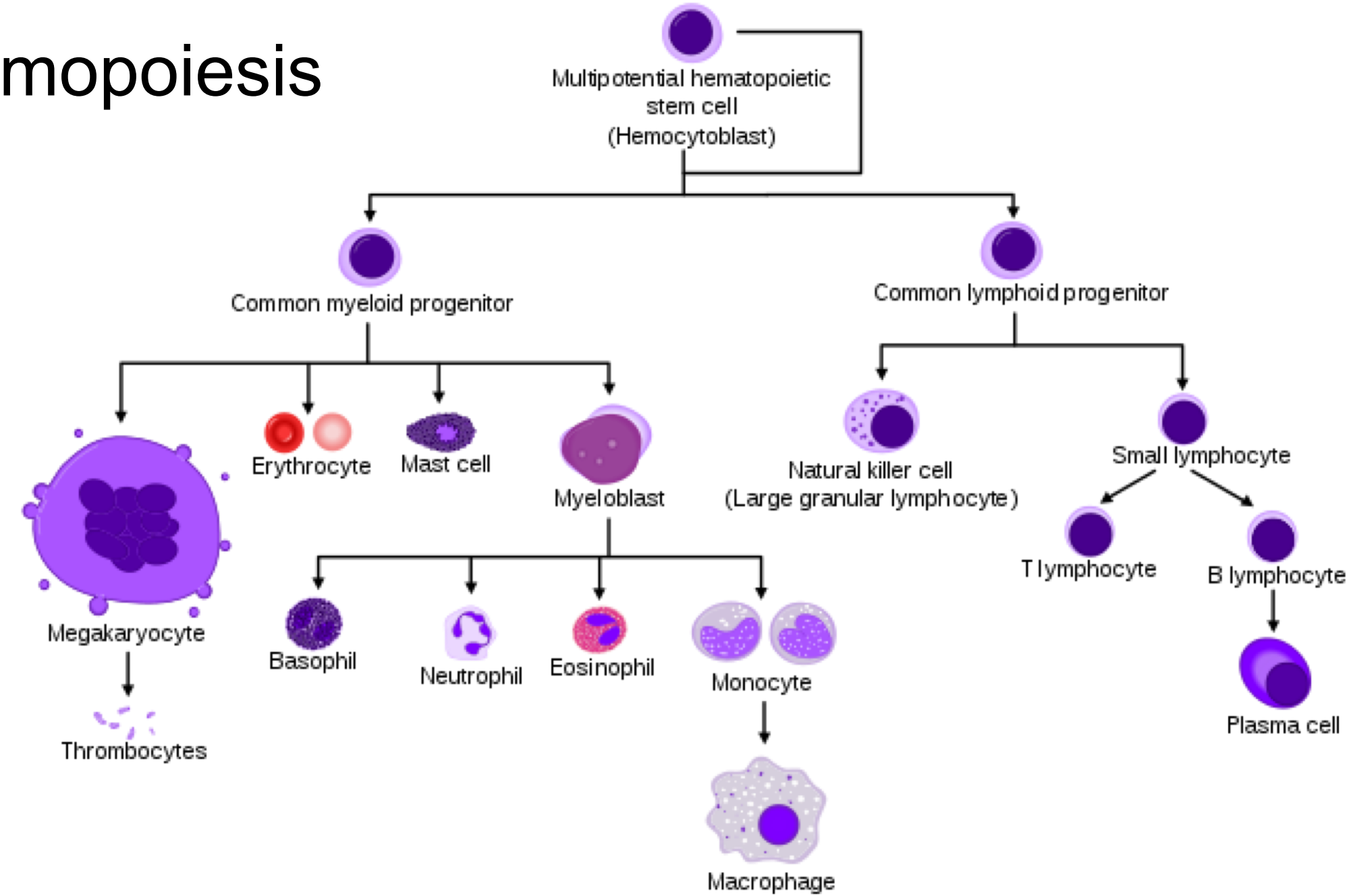
Éva Fődi MD
Hungarian National Blood Transfusion Service
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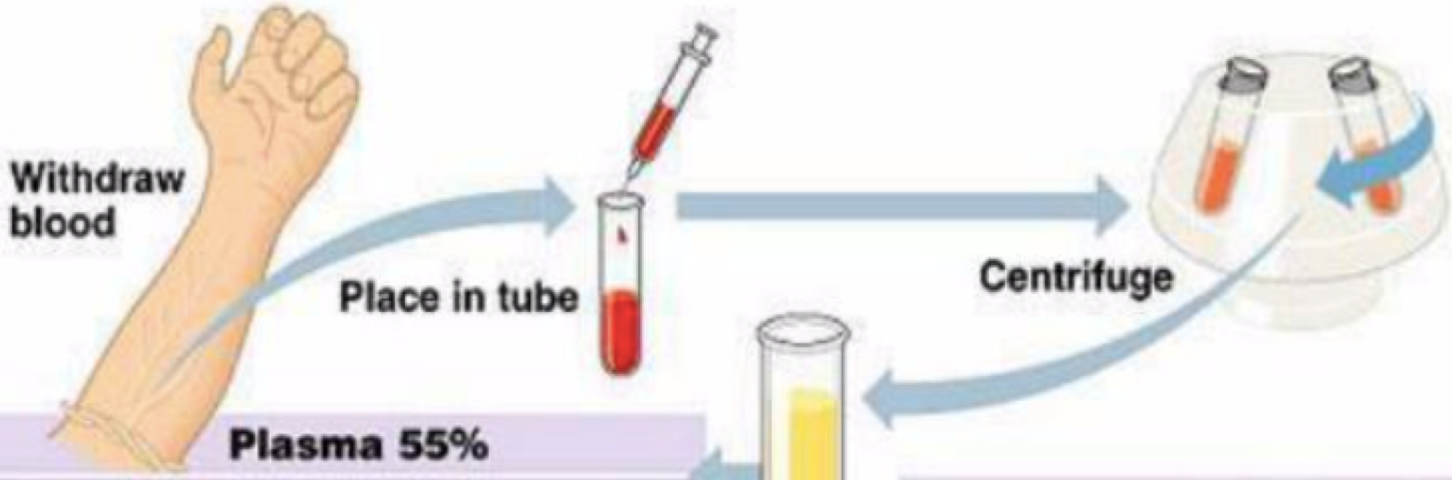
The blood



- Fluid connective tissue:
  - Provides the cells with oxygen and removes carbon dioxide
  - Transports nutrients and hormones
  - Regulates body temperature
  - Brings waste products to the kidneys and liver
  - Contains componants of the immune system and hemostasis

Hemopoiesis





hematocrit

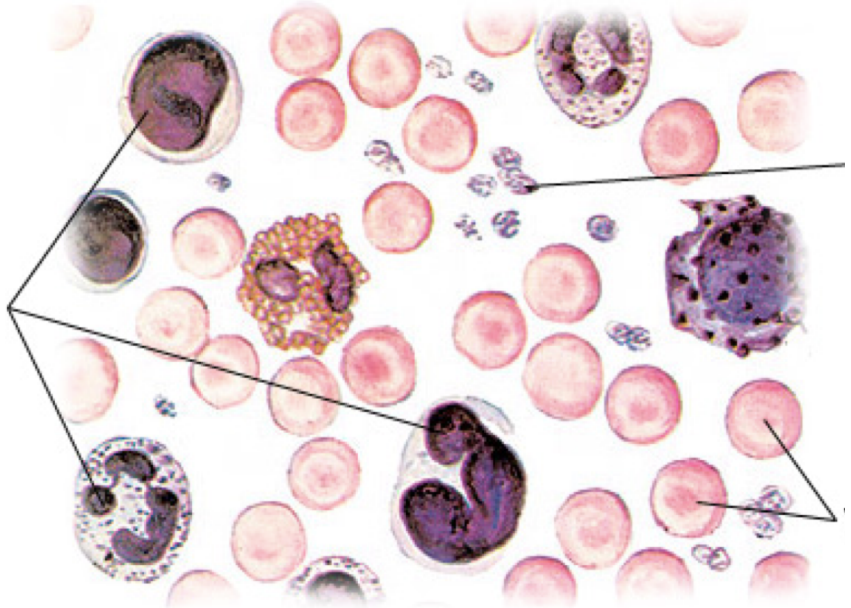
Plasma 55%

Constituent	Major functions
Water	Solvent for carrying other substances
Ions Sodium Potassium Calcium Magnesium Chloride Bicarbonate	Osmotic balance, pH buffering, and regulation of membrane permeability
Plasma proteins Albumin Fibrinogen Immunoglobulins (antibodies)	Osmotic balance pH buffering Clotting Defense
Substances transported by blood Nutrients (e.g., glucose, fatty acids, vitamins) Waste products of metabolism Respiratory gases (O_2 and CO_2) Hormones	

Cellular elements 45%

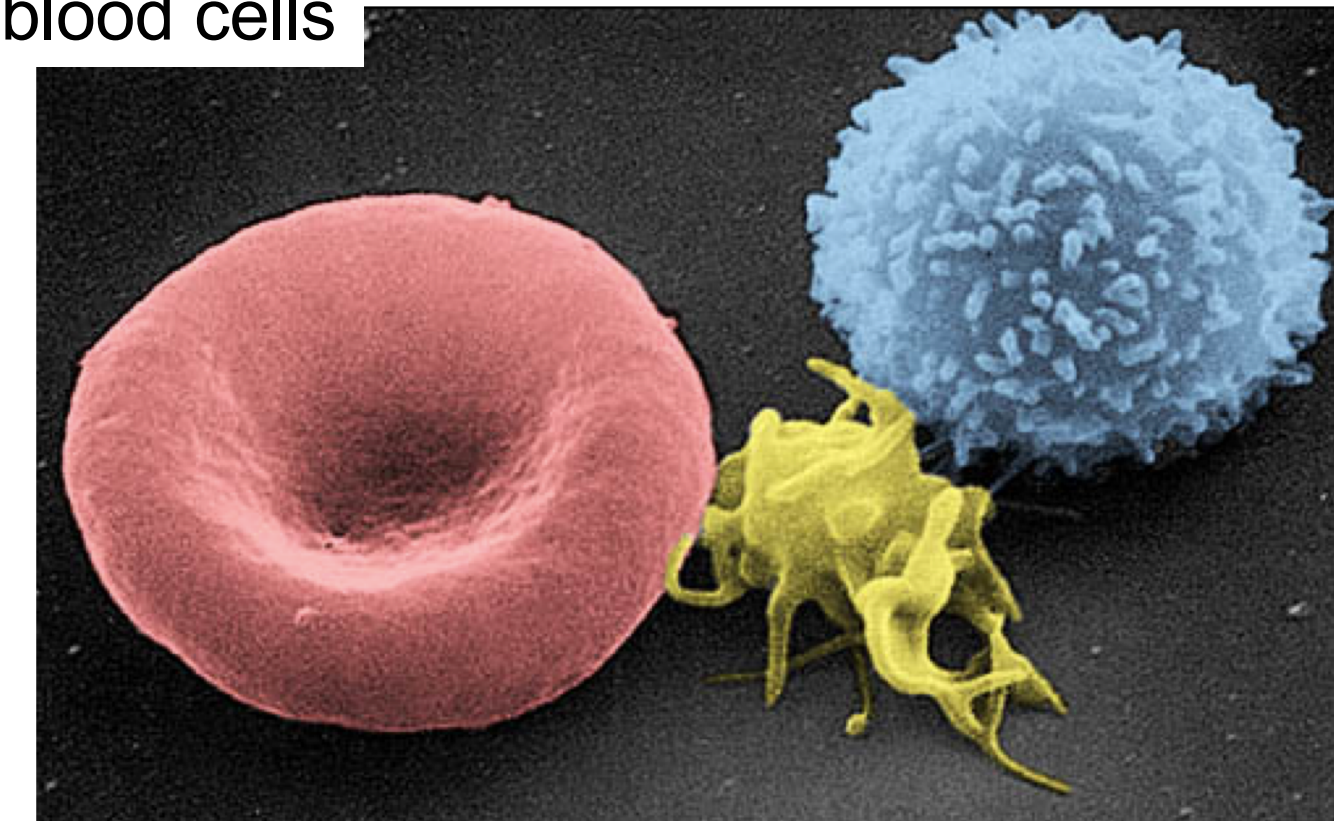
Cell type	Number (per mm^3 of blood)	Functions
Erythrocytes (red blood cells)	5–6 million	Transport oxygen and help transport carbon dioxide
Leukocytes (white blood cells) Basophil Eosinophil Neutrophil Lymphocyte Monocyte	5000–10,000	Defense and immunity
Platelets	250,000–400,000	Blood clotting

White blood cells



Platelets

Red blood cells



Blood transfusion

- Blood transfusion is the process of transferring blood products into the patient's circulation intravenously
- We use only components of the blood – replace what's lost
- Blood is usually transfused to provide the means of maintaining oxygenation of the tissues, or to correct bleeding and coagulation disorders

Blood transfusion

- Transfusions of blood products are associated with several complications, many of which can be grouped as immunological or infectious
- Non-self antigens on blood cells can activate the immune system

The immune system

- The purpose of the human immune system is to defend the body against non-self pathogens or antigens
    - Antigen = any substance that is able to cause an immune response
    - Immunological tolerance against self antigens
- Innate and adaptive immunity
- Humoral and cellular mechanisms within adaptive immune system

Innate immunity	Adaptive (acquired) immunity
Already „works” at birth	Developed after birth
Monocytes/macrophages, granulocytes, dendritic cells, natural killer cells, complement system	B and T lymphocytes
Fast response – hours Immediate maximum response	Longer response – days, weeks lag time between exposure and maximal response
General protection, not antigen-specific	Highly specific for a particular pathogen, antigen-specific
No immunological memory	Improves with each successive exposure = immunological memory

Humoral immune response

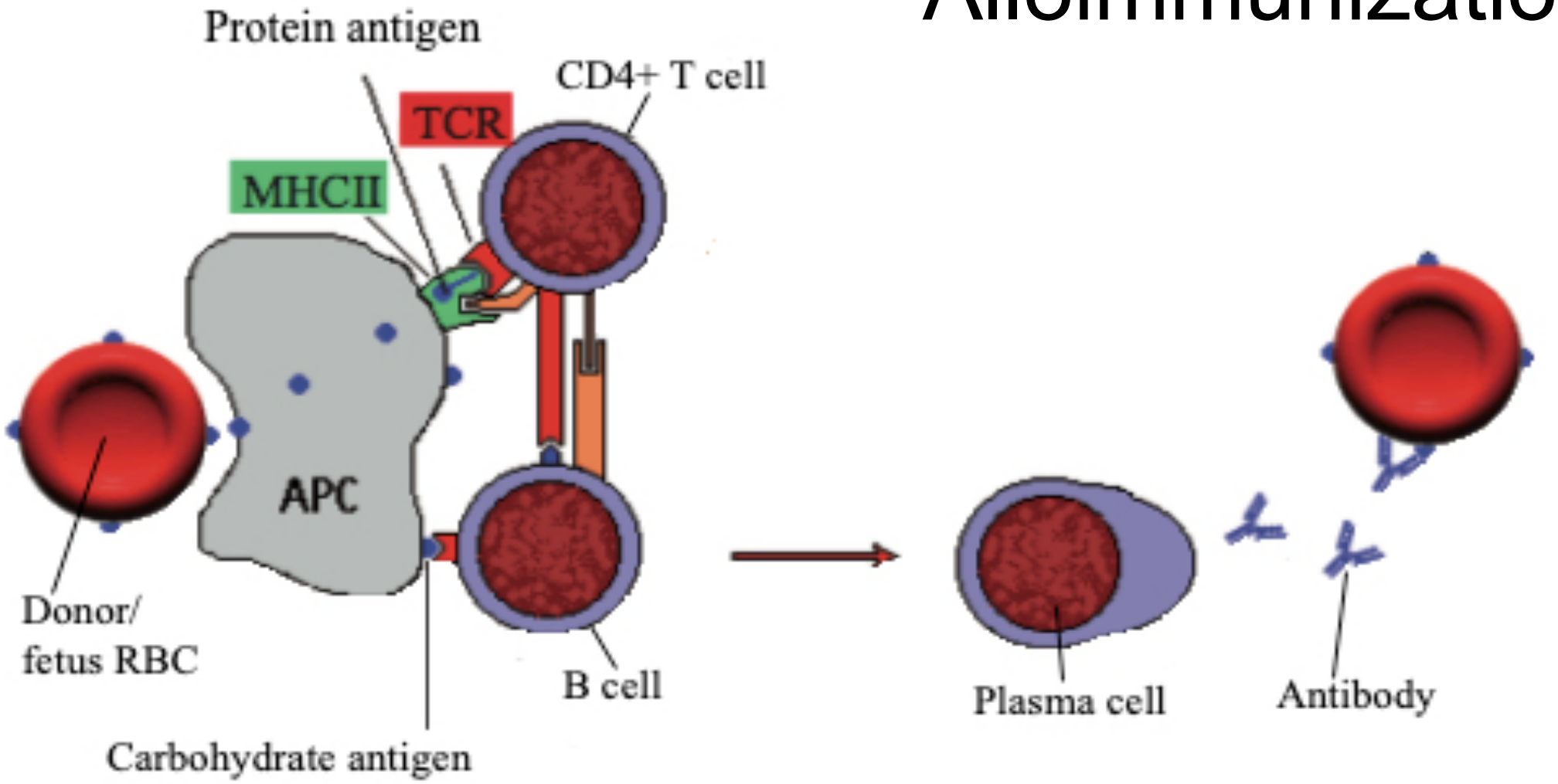
- Humoral immunity dominates in transfusion medicine
  - producing **antibodies** against non-self antigens
  - effector mechanisms are antibody-mediated
- Most important non-self antigens are on the red blood cells

Alloimmunization

- = an immune response to non-self antigens from members of the same species
- Two ways to have unknown RBCs in the circulation:
  - Transfusion – donor's (transfused) cells
  - Pregnancy – fetus's cells
- antibodies formed during alloimmunization are named alloantibodies
  - e.g.: RhD negative patient gets RhD positive blood product, or RhD negative gravida has RhD positive fetus $\longrightarrow$ form anti-D alloantibodies

MHC: major histocompatibility complex

Alloimmunization



Memory cells

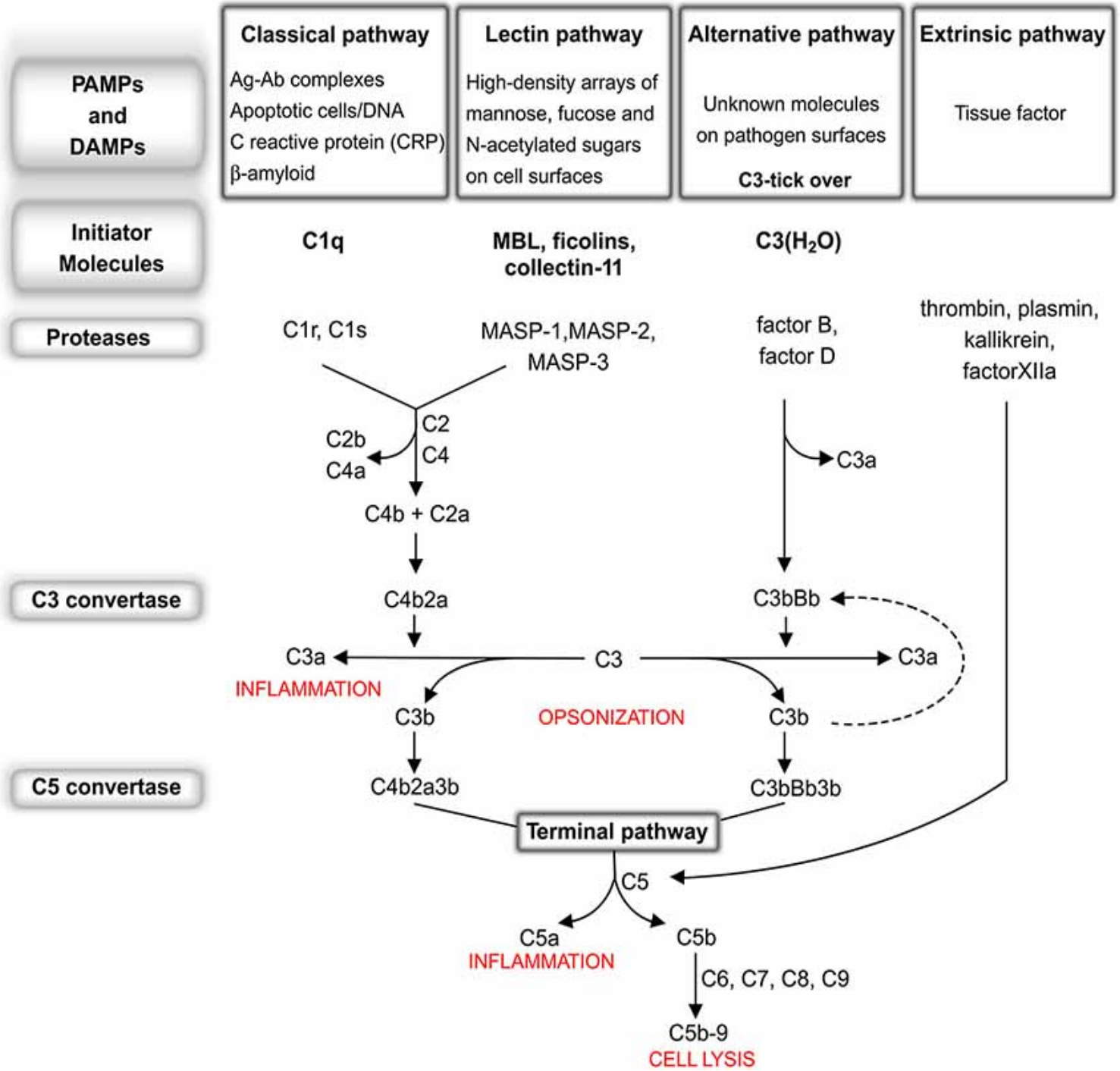
- Formed from B cells
- Play an important role in adaptive immunity
- The immune system is ”able to remember” previous immune responses
  - the answer improves and becomes more efficient after each exposure

Effector mechanisms of antibody-mediated immune response

- Neutralization
- Lysis by complement system activation
- Phagocytosis
- Antibody-dependant cellular cytotoxicity (ADCC)

Complement system

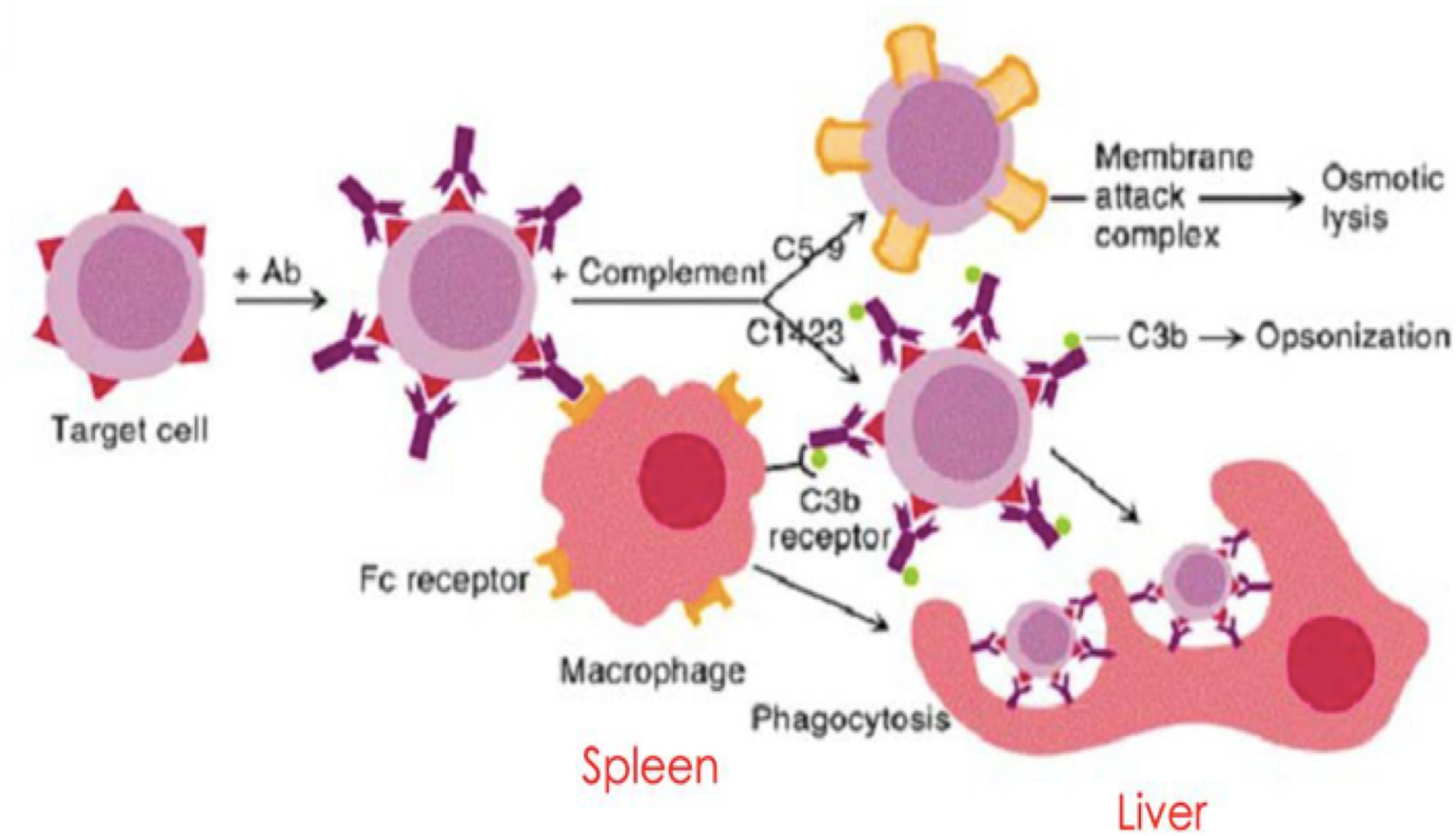
- Enzyme cascade:
  - Inactive proteins, synthesized by the liver and circulate in the blood
- IgM and IgG1, IgG3 antibodies are able to activate the system by the classical pathway
- The end result of *complement activation* or *complement fixation* cascade:
  - stimulation of phagocytes to clear foreign and damaged material
  - inflammation to attract additional phagocytes
  - activation of the cell-killing membrane attack complex = MAC intravascular cell lysis


Classical pathway
Ag-Ab complexes
Apoptotic cells/DNA
C reactive protein (CRP)
β-amyloid
Lectin pathway
High-density arrays of mannose, fucose and N-acetylated sugars on cell surfaces
Alternative pathway
Unknown molecules on pathogen surfaces
C3-tick over
Extrinsic pathway
Tissue factor
PAMPs and DAMPs
Initiator Molecules
C1q
MBL, ficolins, collectin-11
C3(H2O)
Proteases
C1r, C1s
MASP-1,MASP-2, MASP-3
factor B, factor D
thrombin, plasmin, kallikrein, factorXIIa
C2
C4
C2b
C4a
C4b + C2a
C3a
C3 convertase
C4b2a
C3bBb
C3a
C3
C3a
INFLAMMATION
OPSONIZATION
C3b
C3b
C5 convertase
C4b2a3b
C3bBb3b
Terminal pathway
C5
C5a
INFLAMMATION
C5b
C6, C7, C8, C9
C5b-9
CELL LYSIS

Phagocytosis

- phagocytosis is caused by IgG antibodies that are unable to activate the complement system (or only up to C3d)
- Red blood cells are coated with specific antibodies (produced during immunisation) and are destroyed by macrophages in the spleen and liver
- Cell lysis is extravascular

Antibody-Coated RBCs are Destroyed by Macrophages, Mostly in the Spleen



Primary immune response

- The immune system contacts an antigen for the first time
  - IgM is the first antibody to appear
  - IgG antibodies begin to produce a few days later
  - Immunoglobulin class switching = isotype switching
    - IgM $\longrightarrow$ IgG

Secondary immune response

- The immune system contacts an antigen for the second and subsequent times
- IgG appears earlier, and its final concentration is higher than in the primary response

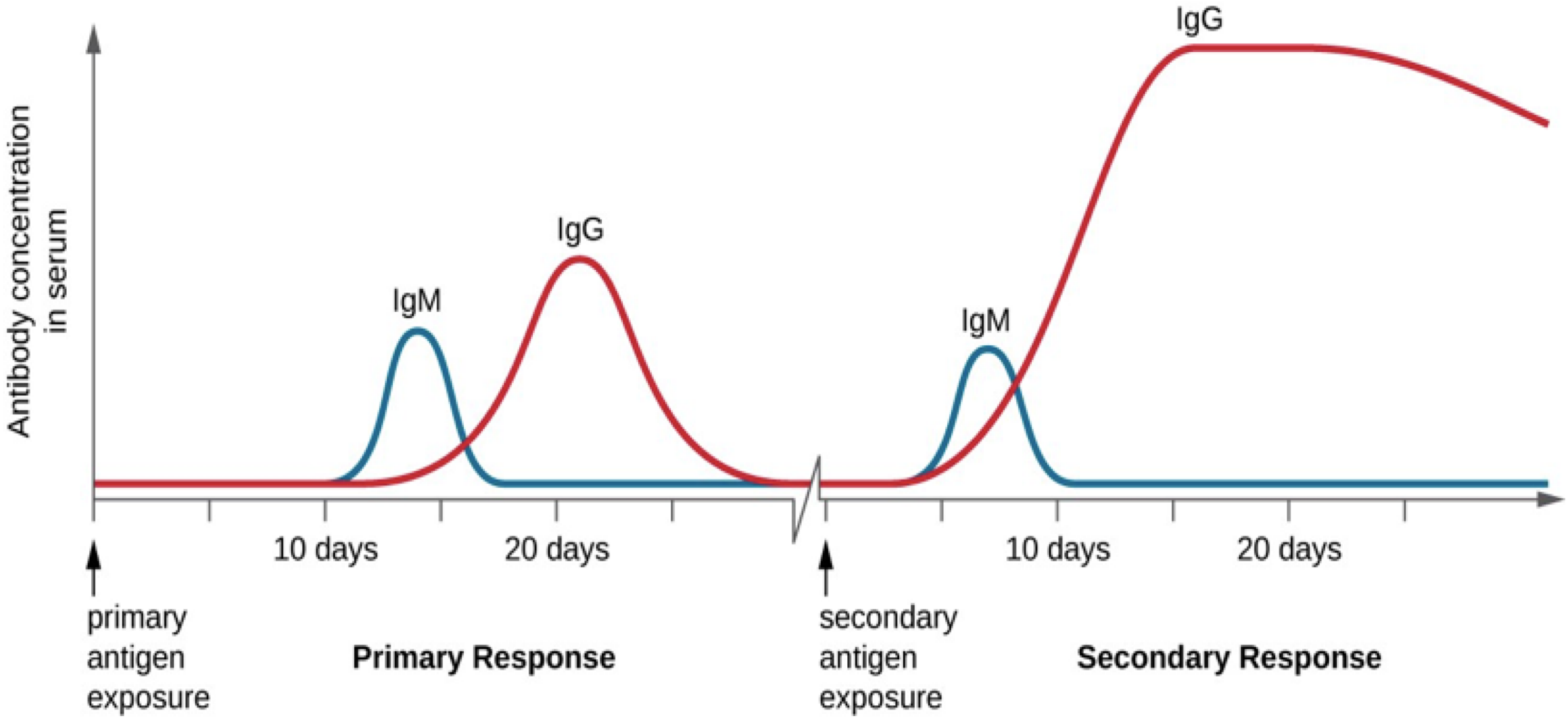


Figure 4. Compared to the primary response, the secondary antibody response occurs more quickly and produces antibody levels that are higher and more sustained. The secondary response mostly involves IgG.

In vitro antigen-antibody reactions, basics of blood group serology

Blood group serology

- The goal is to provide compatible blood products to the patient
  - Transfusion must be effective
  - Donor cells must show normal survival in recipient's circulation
- Serological compatibility means that there is no antibody in the recipient's serum to antigen on the donor's red blood cell

Blood group serology

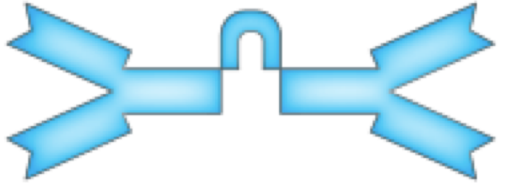
- Serological compatibility testing:
  - Laboratory blood group test:
    - ABO typing (forward + reverse group)
    - RhD typing
    - Antibody screening
    - Direct antiglobulin test (DAT)
  - Laboratory crossmatch

Antibodies = immunoglobulins

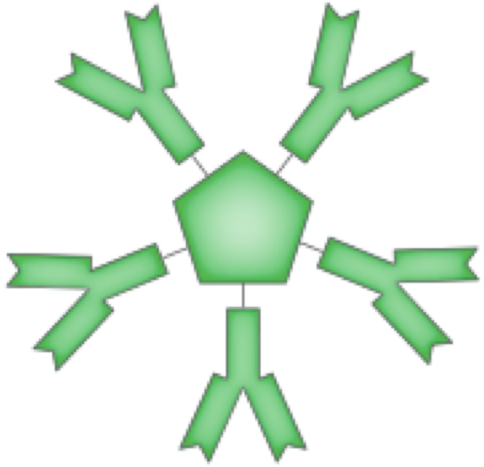
- IgA, IgD, IgE, IgG, IgM
- Glycoproteins – produced by plasma cells
- They bind to specific antigens



Monomer
IgD, IgE, IgG

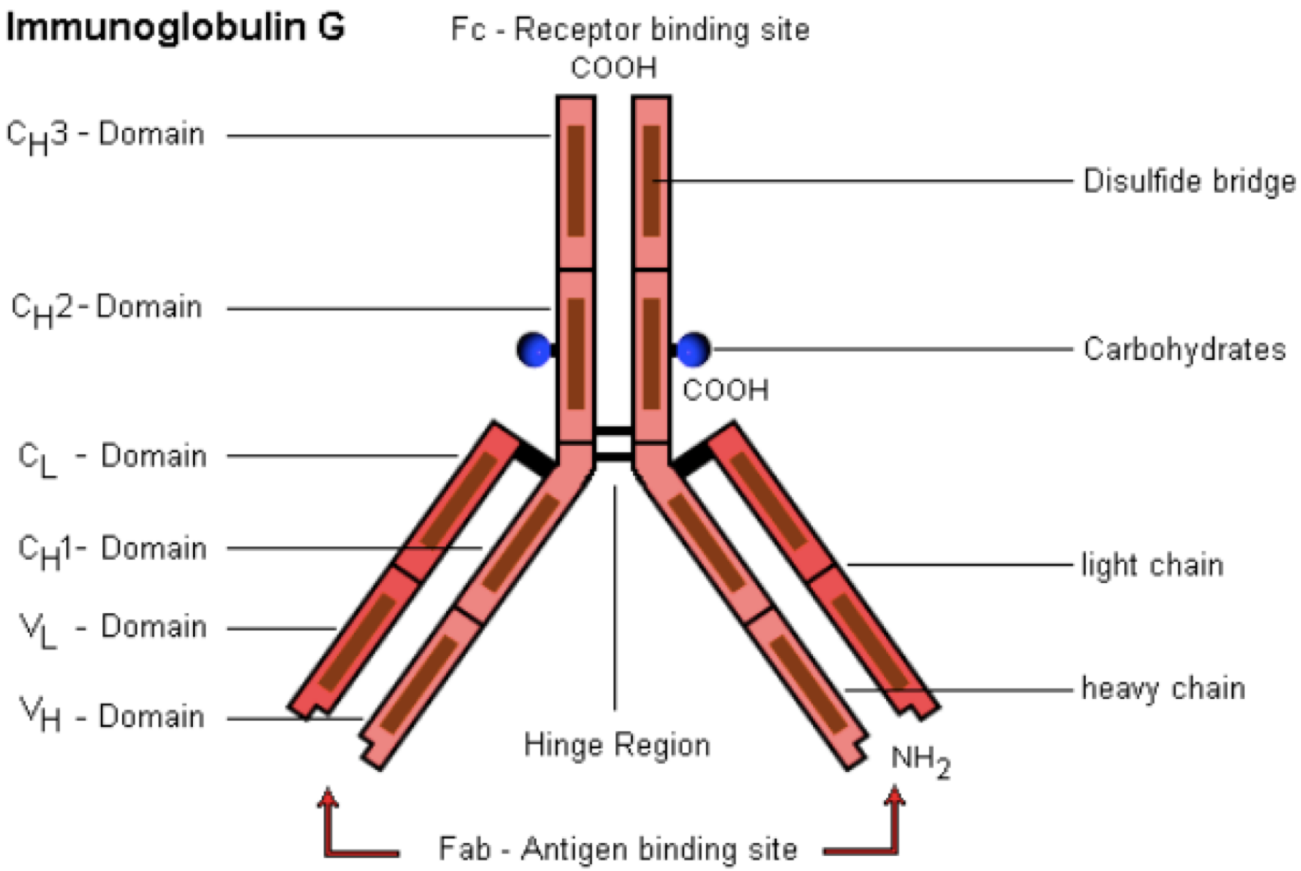


Dimer
IgA



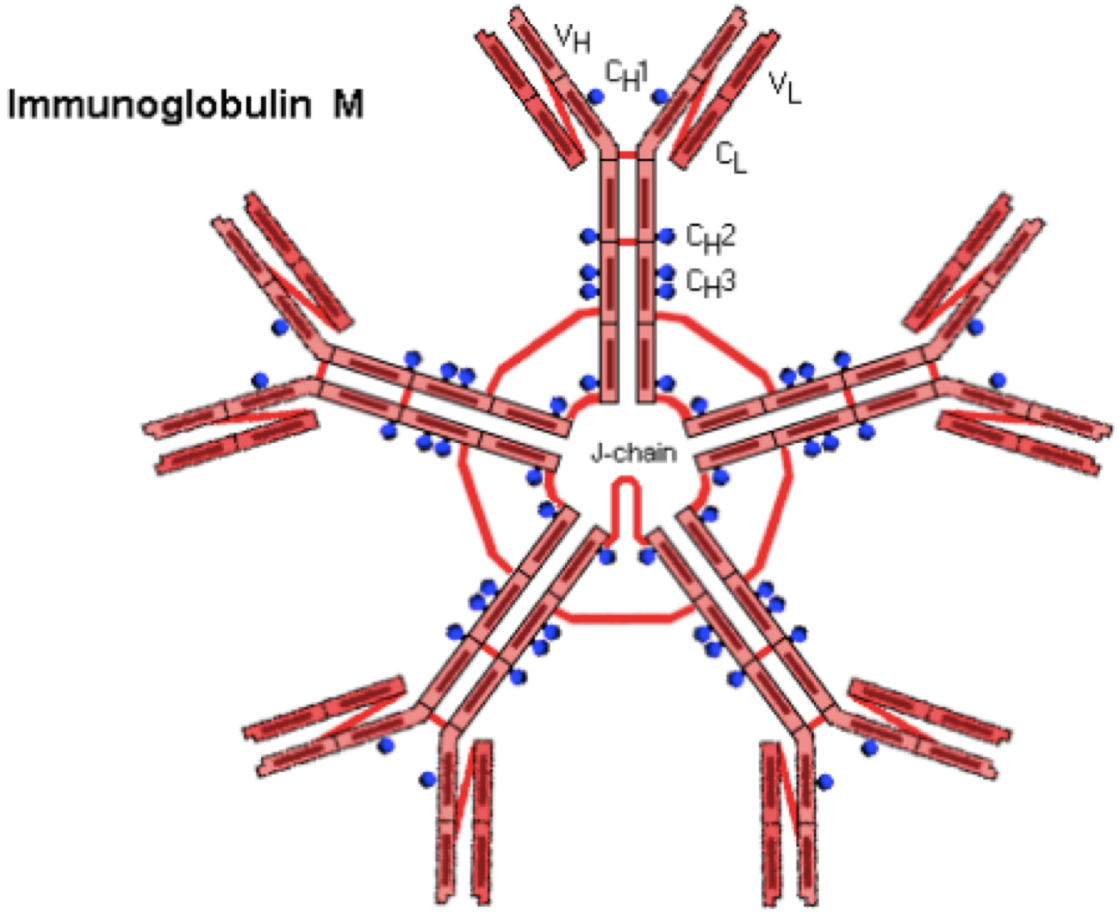
Pentamer
IgM

IgG



- Accounting for 70-75% of human immunoglobulins
- Classified into 4 subclasses: IgG1, IgG2, IgG3 and IgG4
  - IgG1 and IgG3 can activate the complement system
- Transferred to the fetus through the placenta
- Indirect agglutination
- React optimally at 37 ˚C – warm antibody

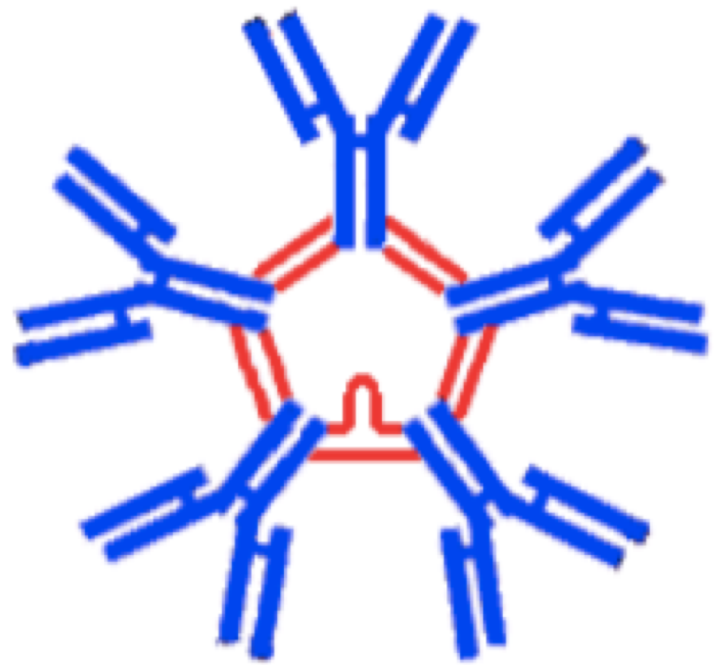
IgM



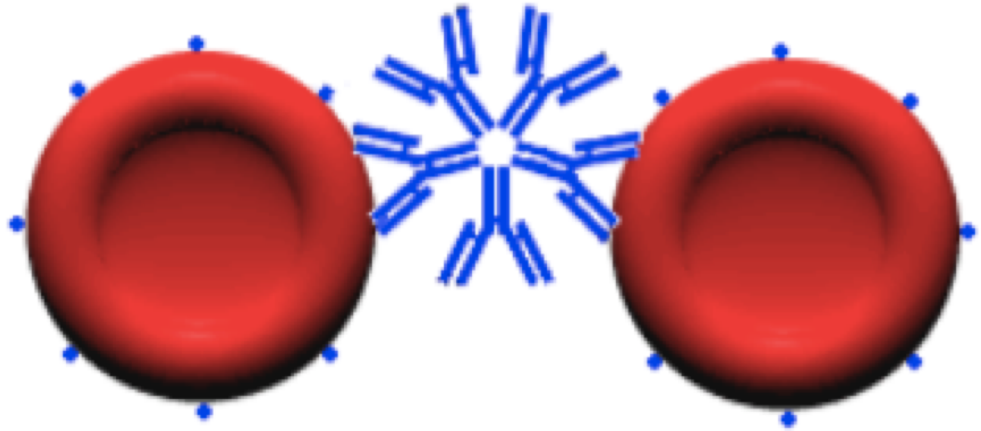
- accounting for about 10% of human immunoglobulins
- Complement system activation
- Direct agglutination
- Active at room temperature, react optimally at 4 ˚C – cold antibody



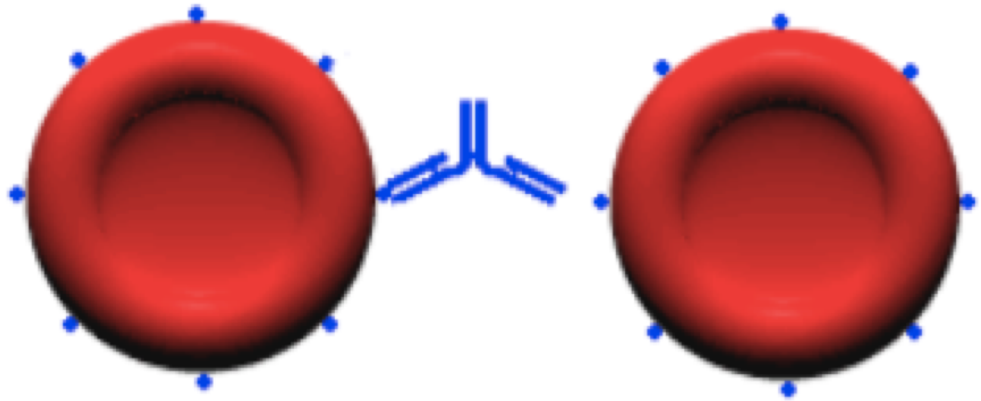
IgG



IgM



Complete Antibody (IgM)



Incomplete Antibody (IgG)

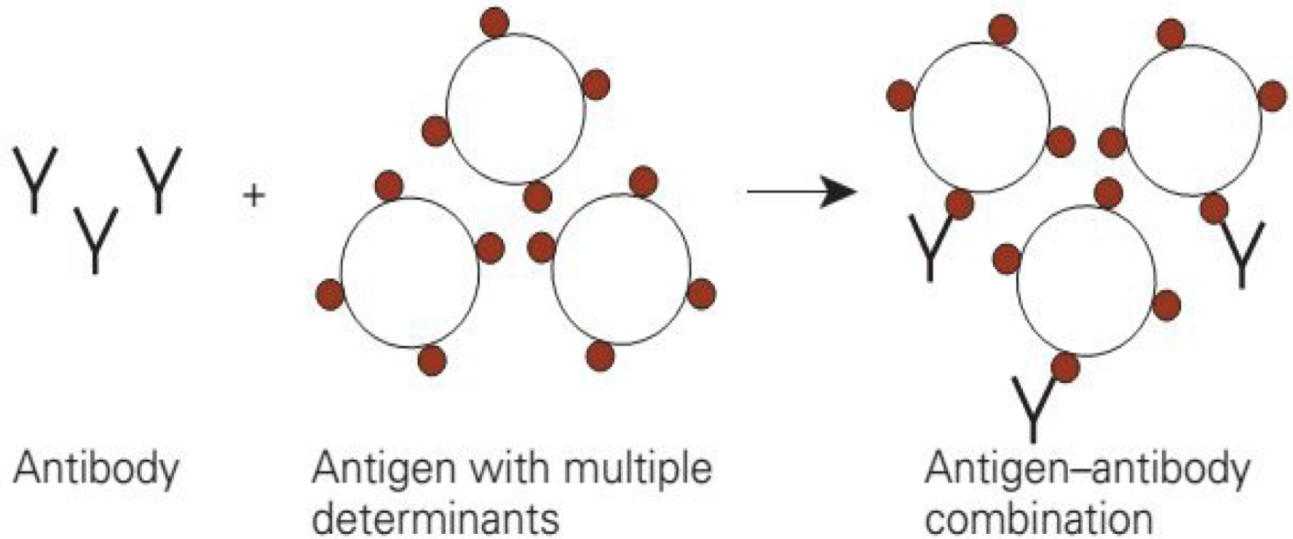
Serological tests

- Based on antigen-antibody reactions
- In vitro antigen-antibody reactions:
  - **Agglutination**
  - Hemolysis
  - Absorption
  - Elution

Agglutination

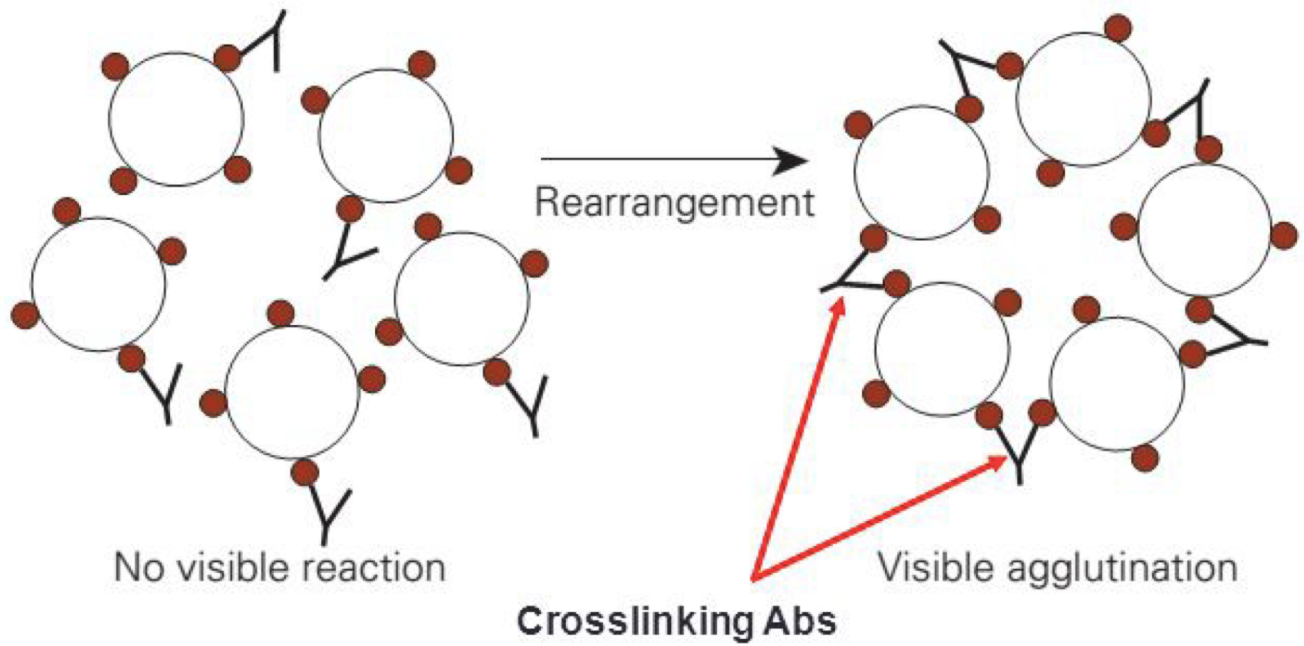
- Agglutination is a two-step process that results in the formation of a stable lattice network:
- 1. Sensitization:
  - Antigen+antibody complex
  - Binding through single antigenic determinant
- 2. Lattice formation:
  - The formation of crosslinks creates visible aggregates = agglutination
  - Binding through multiple antigenic determinants

Sensitization



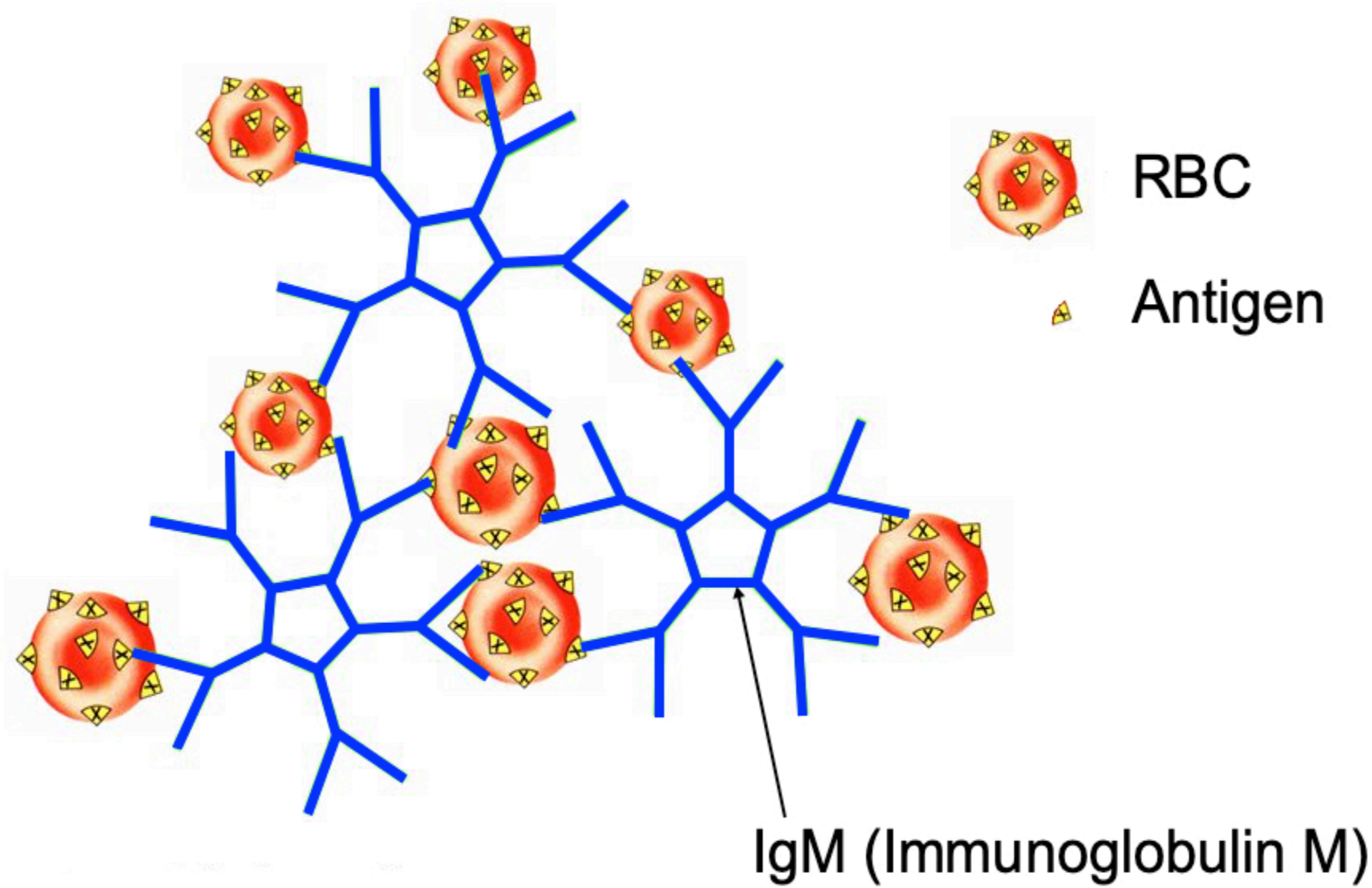
- Antibody molecules attach to their corresponding Antigenic site (epitope) on the particle
- There is no visible clumping

Lattice formation

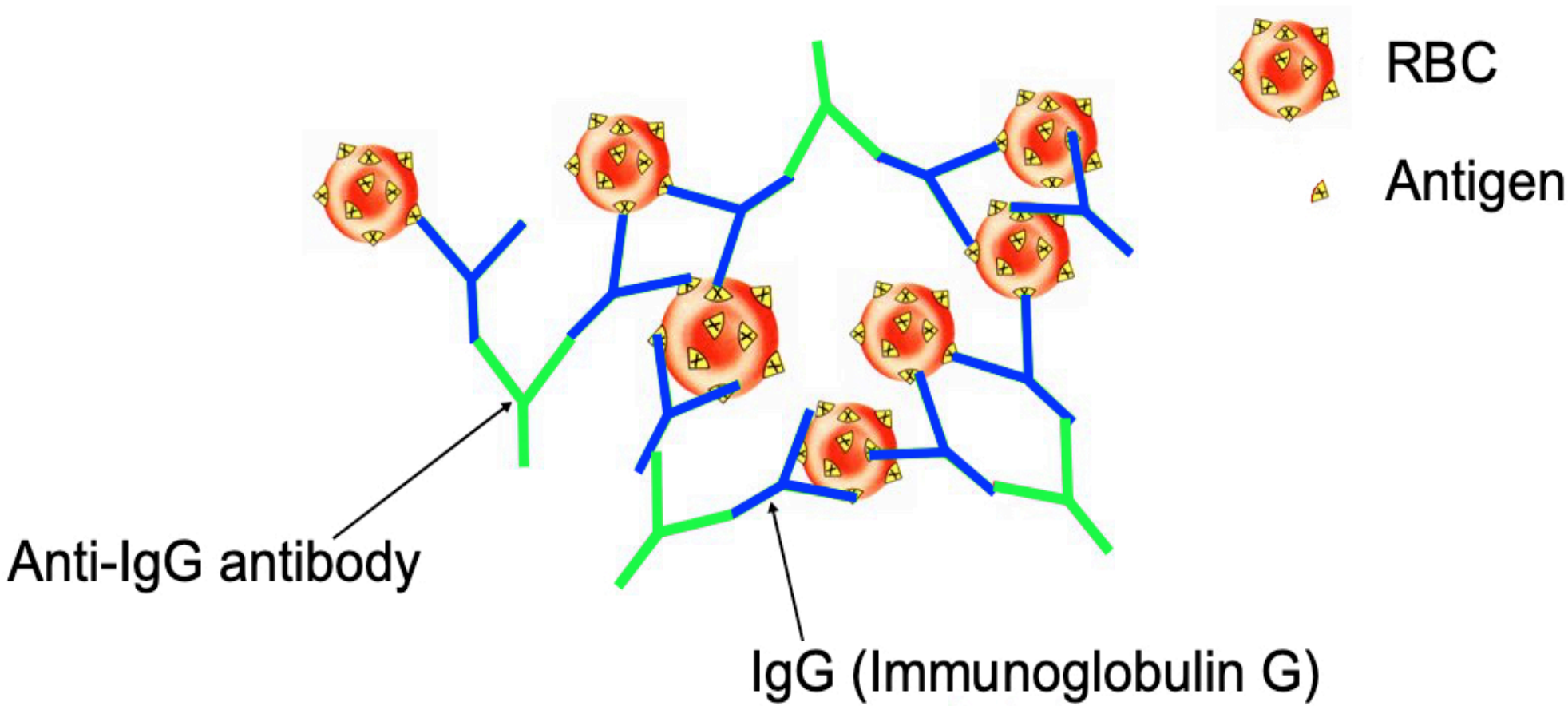


- Antibody molecules crosslink the particles forming a lattice that results in visible clumping or agglutination

Direct agglutination

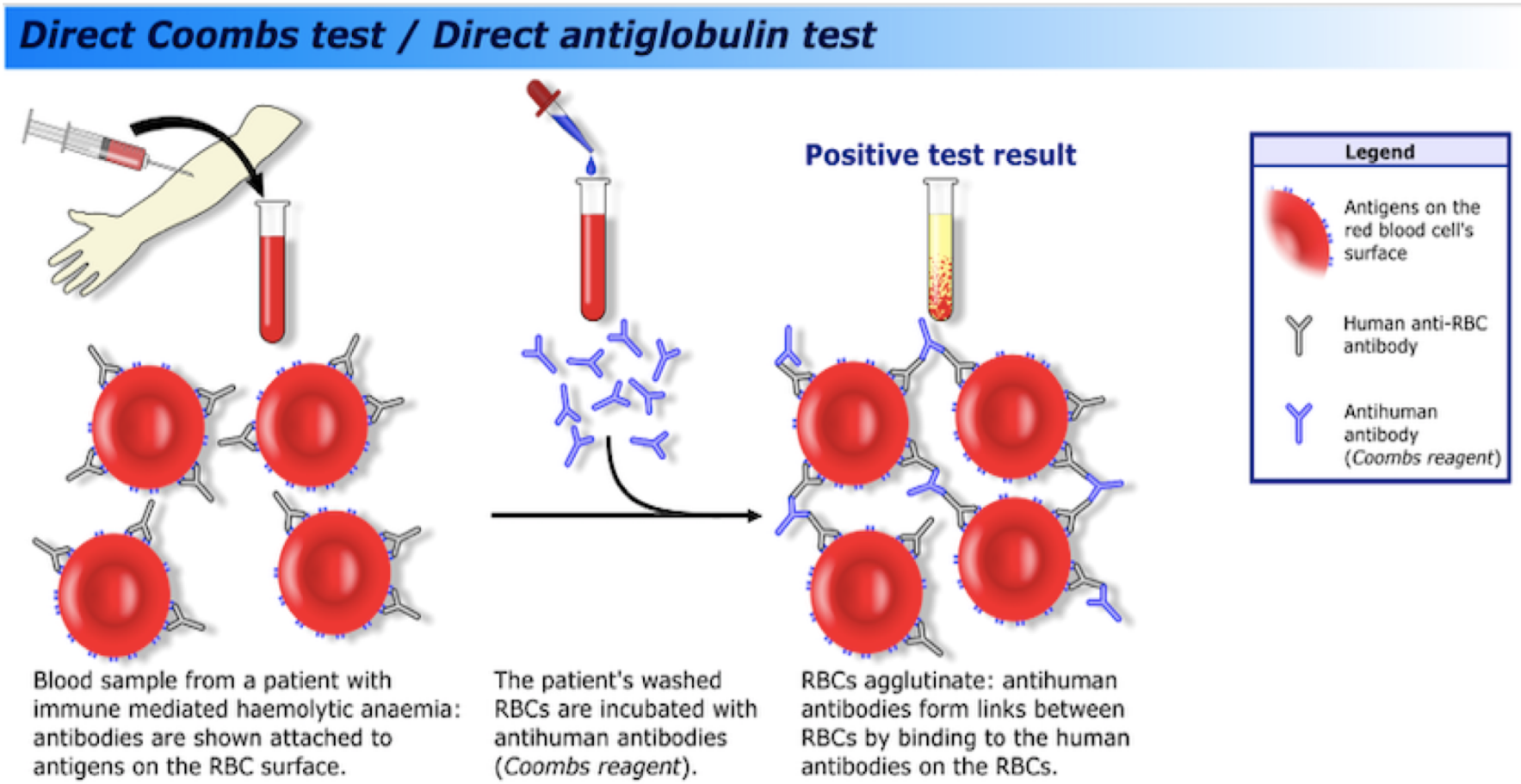


Indirect agglutination



DAT= direct antiglobulin test

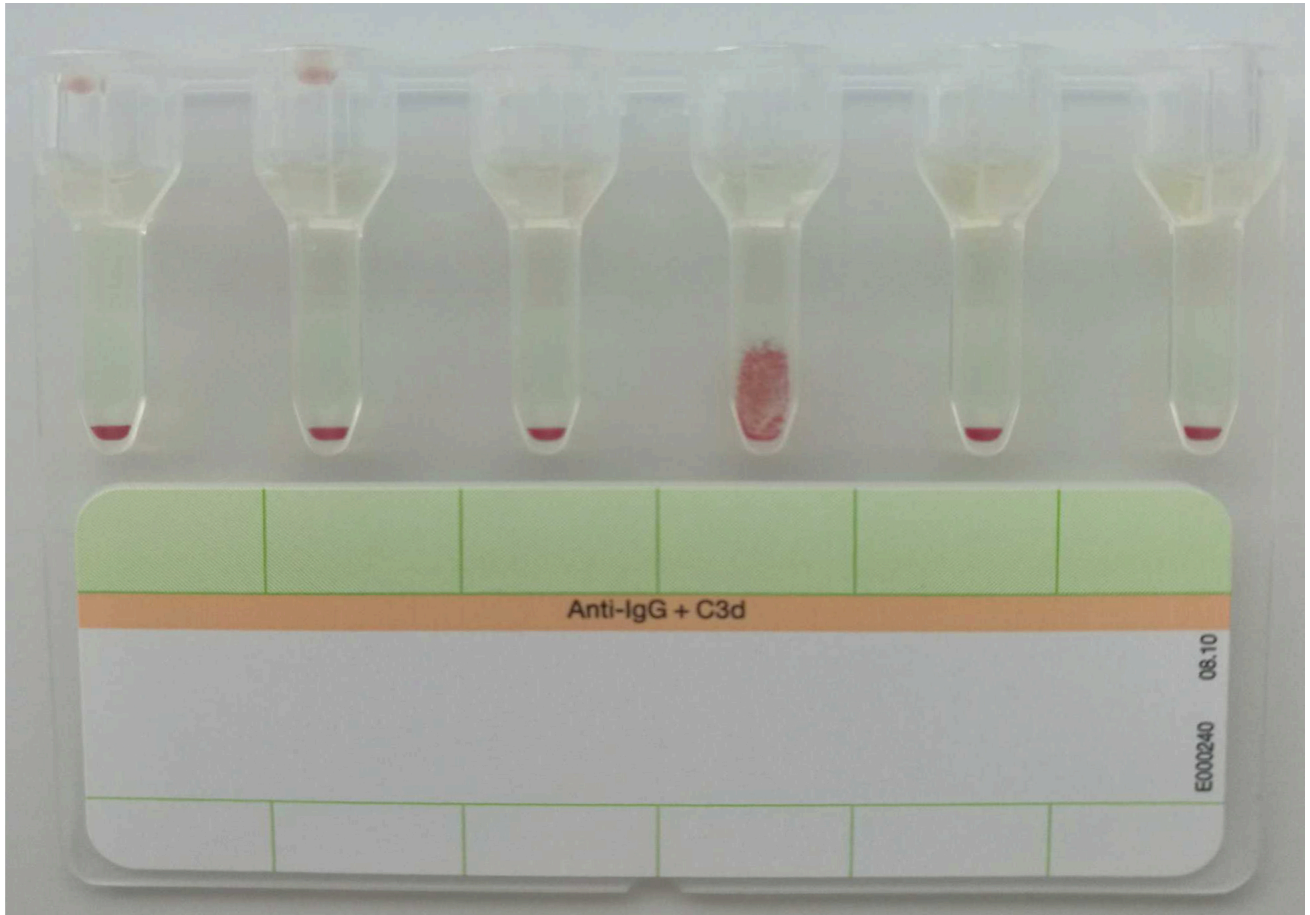
The red blood cells of the recipient may be covered by antibodies and/or complements



DAT

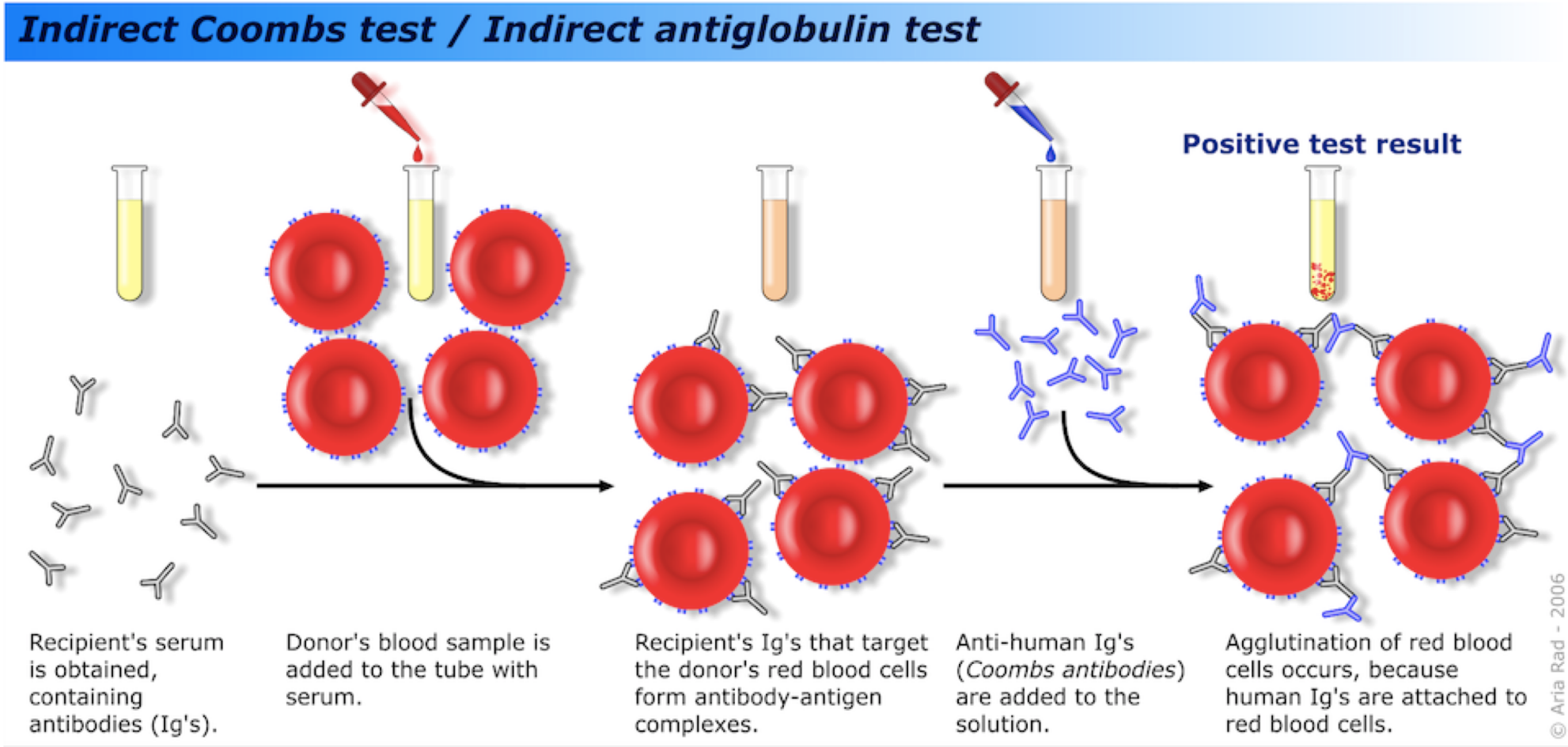
Coombs reagent and gel card contain anti-IgG+C3d





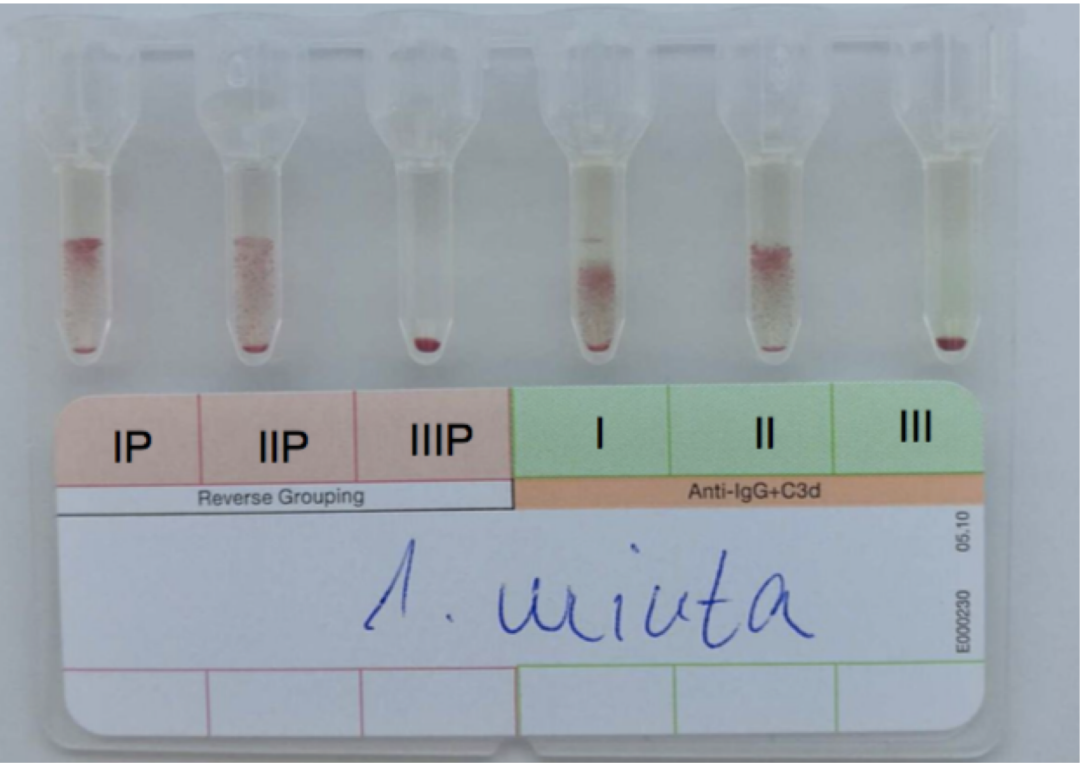
Antibody screening

- Detects **alloantibodies** in recipient's serum
- Indirect antiglobulin test



Antibody screening and identification

- We use test RBCs from group O donors:
  - Test cell reagents are specially made for antibody screening and identification by different laboratories
  - The manufacturer includes an antigen table that shows the important blood group phenotypes of test cells
- Two media are used:
  - LISS/AGT - low-ionic-strength saline with indirect antiglobulin test
  - Enzyme – papain or bromeline











Antibody screening

- Column agglutination technique
- Gel card
- 50 µl test cells + 25 µl recipient serum -
- Incubation at 37 °C for 15 min
- Centrifugation
- Interpretation

Antibody identification

- If the antibody screen is positive
- The specificity of the antibody is identified by testing the serum against a panel of 11 Group O RBCs of varying phenotypes
  - The pattern of positive and negative reactions with these cells identifies the antigen against which the antibody is directed



LISS/AGT test



Papain test

BIO-RAD

Set ID-DiaPanel: 45161.25.x (Japan: 4516.25.xx)
Set ID-DiaPanel P: 45171.25.x (Japan: 4517.25.xx)

LOT 06171.25.x – 06271.25.x (Japan: 0617.25.xx – 0627.25.xx)
05361.25.x – 05461.25.x (Japan: 0536.25.xx – 0546.25.xx)

2018.01.29 (Japan: 29.01.18)
V.I.P. Software: P39

Antigen-Tabelle / Antigen-Table / Table d'antigènes / Tabella antigenica / Tabla de antígenos / Tabela de antígenios
Antikörper-Identifizierung / Antibody identification / Identification d'anticorps / Identificazione anticorpale / Identificación del anticuerpo / Identificação do anticorpos

	Rh-hr	Möglicher Genotyp / Probable Genotype / Genotype probable / Probabile genotipo / Genotipo probable / Genótipo provável	Spender / Donor / Donneur / Donatore / Donante / Dador	Rh-hr						Kell						Duffy		Kidd		Lewis		P	MNS				Luth.		Xg		Spez. Antigene / Special types / Antigènes part. / Antigeni particolari / Otros Antígenos / Tipos especiais	
				D	C	E	c	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a	♀ ♂		
1	$CCC^wD.ee$	$R_1{}^wR_1$	243366	+	+	0	0	+	+	0	+	0	+	nt	nt	+	0	0	+	0	0	+	+	+	0	+	0	+	+			1
2	CCD.ee	R_1R_1	639208	+	+	0	0	+	0	+	+	0	+	nt	nt	0	+	+	+	0	0	0	0	+	0	+	0	+	nt			2
3	ccD.EE	R_2R_2	758532	+	0	+	+	0	0	0	+	0	+	nt	nt	+	0	+	+	+	0	0	+	0	+	0	0	+	0		Bg(a+)	3
4	Ccddee	r′r	431240	0	+	0	+	+	0	0	+	0	+	nt	nt	0	+	+	0	0	+	0	0	+	0	+	0	+	0			4
5	ccddEe	r″r	143075	0	0	+	+	+	0	0	+	0	+	nt	nt	+	0	+	0	0	+	+	0	+	0	+	0	+	+			5
6	ccddee	rr	106860	0	0	0	+	+	0	+	+	0	+	nt	nt	0	+	+	0	0	+	+	+	0	+	0	0	+	+			6
7	ccddee	rr	185699	0	0	0	+	+	0	0	+	+	+	nt	nt	0	+	0	+	0	+	0	0	+	+	0	0	+	+		Bg(a+)	7
8	ccD.ee	Ror	073982	+	0	0	+	+	0	0	+	0	+	nt	nt	0	0	+	+	0	0	+	+	+	+	0	0	+	nt			8
9	ccddee	rr	857188	0	0	0	+	+	0	0	+	0	+	nt	nt	0	+	0	+	+	0	+	+	+	0	+	+	+	0			9
10	ccddee	rr	550449	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	0	+	0	+	+	+	0	+	0	0	+	+		*Bg(a+)*	10
11	ccddee	rr	132258	0	0	0	+	+	0	0	+	0	+	nt	nt	+	0	+	0	0	+	+	+	0	0	+	0	+	+			11
	Patient / Patient / Patient / Paziente / Paciente / Paciente																													Eigenkontrolle / Autocontrol / Autocontrôle / Autocontrollo / Autocontrol / Auto-controlo		

Anti-E antibody

Resultat / Risultato / Re

LISS / Coombs	E

Anmerkungen siehe rückseitig / Remarks see overleaf / Voir les remarques au verso / Per le note consultare il retro / Ver observaciones en el reverso / Ver observações no verso

Name / Name / Nom / Nome / Nombre / Nome

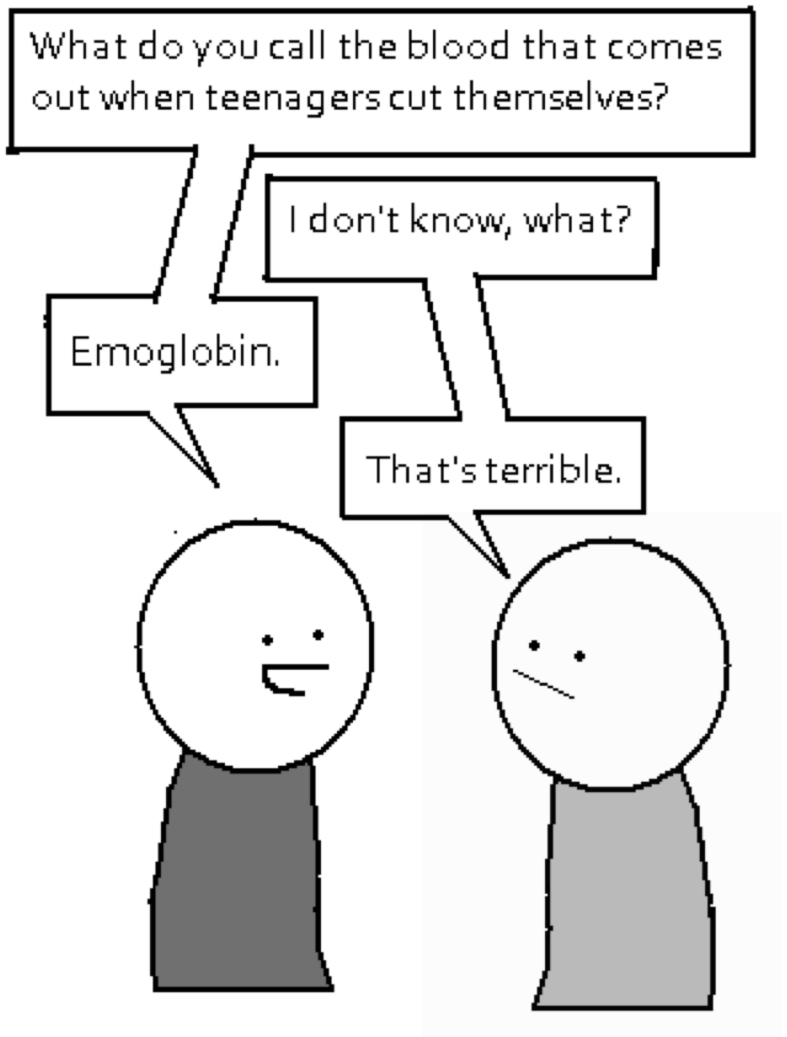
Blutgruppe + Antigene / Blood group + antigens /

Recipients with antibodies

- The identified antibody appears at the patient's immunohematological status in our IT system
- Regulate further transfusions:
  - For a patient who has a clinically significant antibody (or antibodies), the units selected for crossmatch will be antigen-negative
    - e.g.: the patient forms anti-E alloantibodies, he must be transfused with E antigen negative red cell concentrate
  - Transfused red cells have to shown normal survival in the patient’s circulation
  - This regulation also protects the patient from additional immune responses caused by the E antigen

Crossmatch

- Crossmatching is a method of confirming compatibility between the patient and the donor
  - Modelling the transfusion in vitro
  - 25 ul recipient serum + 50 ul donor RBCs in indirect antiglobulin technique (gel card)
- Negative reaction = red cell concentrate is compatible
- Positive reaction = red cell concentrate is incompatible



Thank you very much for your attention!

If you have any questions, please feel free to contact me: fodi.eva@ovsz.hu or on Coospace.