



University of
BRISTOL



Blood and Transplant

Serology: the lynchpin of erythrocyte research

Carole Green

Bristol Institute for Transfusion Sciences

BBTS Glasgow 2017



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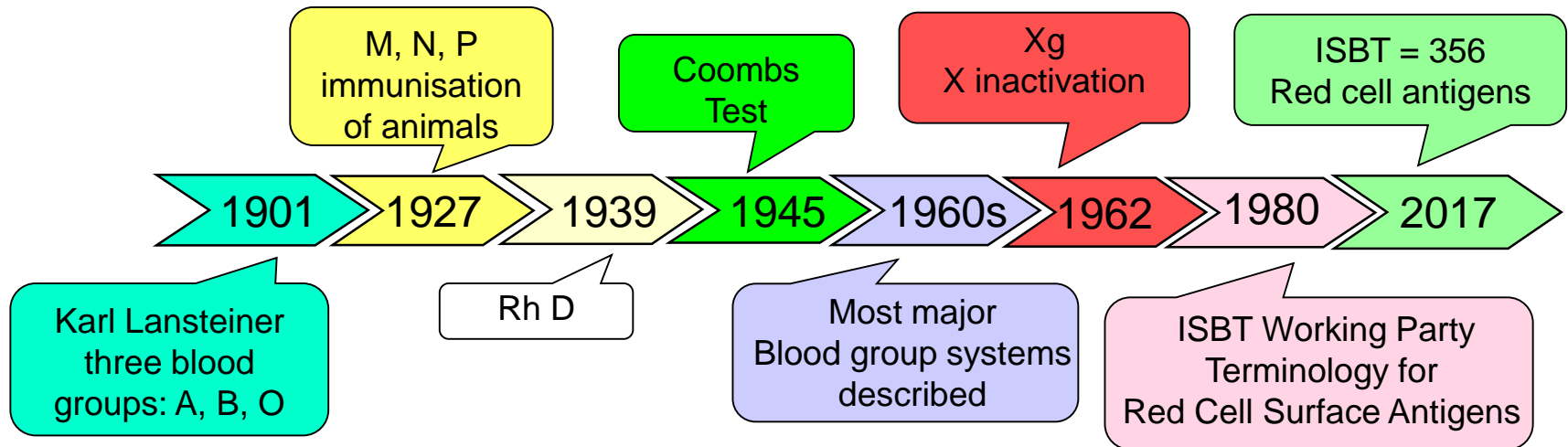
O MsMs P1 rr Lu(a-b+) K-k+ Le(a-b+) Fy(a-b+) Jk(a-b+) Xg(a+^w)

BBTS Glasgow 2017

Blood group serology

- Study of antigens on the surface of red blood cells (RBCs)
- Classified according to antibodies in the plasma and immunological compatibility
- Antibodies may recognise polymorphic, high or low frequency antigens
- The first and, for a time, the only research tool for exploring red cell antigens; initially by direct agglutination and later with the antiglobulin test

Some important serological events



Xg was announced by telegram as **'IT'S SEX'**



International Society
of Blood Transfusion

Facilitating knowledge about transfusion medicine
to serve the interests of donors and patients

ISBT working party on red cell immunogenetics and terminology, Copenhagen 2017

356 antigens

318 belong to 36 systems

Most recent published report:

ISBT working party on red cell immunogenetics and terminology, Seoul, 2014, and London, 2015 (*ISBT Science Series* (2016) 11: 118-122)

Established 1980 to bring order to the chaos

Techniques in serology

Techniques vary, all rely on the ability of antibodies (human, animal, monoclonal) and lectins to agglutinate RBCs

Capillary



Methods

- Glass slide
- Tube
- Capillary
- Gel cards
- Microtitre plates
- Automation

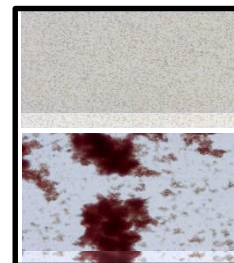
Tools

- Low ionic saline, albumen
- Enzyme and chemical modification
- Antibody Inhibition with recombinant proteins, serum, saliva, urine, pigeon egg white

Microplate



Slide/tube



Gel card



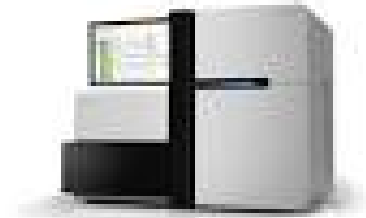
Blood grouping: now and then



Automated blood group analyser



PCR thermocycler



Next generation sequencer



Serology bench 1970

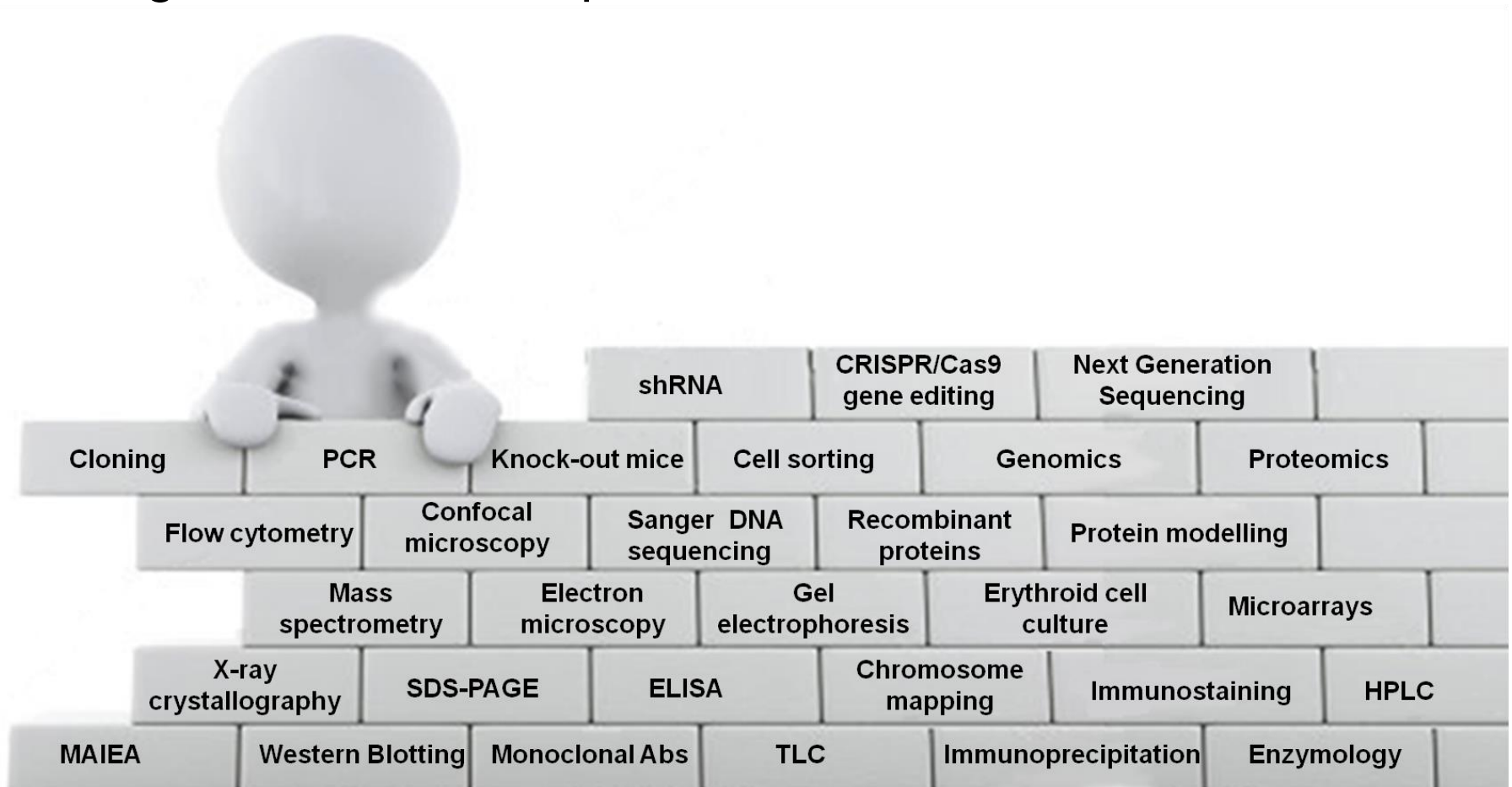
Manual blood grouping continues
Valuable in the reference lab
Subjective
Skilled serologist



**Serology bench 2017
(Reference lab)**

Additional techniques

Essential addition to our battery of serological tests
Application of these has expanded our knowledge of
antigens, their carrier proteins and function



Simple method for Ch/Rg testing

Helico bacter pylori

A4GALT and P₁

LFA ELO

MAIEA (CR1)

Mab H86

En(a-)

CD99/12E7

LFA SHIN

MNS Mg+

Monoclonal antibody production (CR1, CD59, GPA)

RH JAL

CD55 deficiency in Japanese

DWI, a novel "high-grade" partial D

CD44/CD44

RH Tar

RHAG

CROM WES^a WES^b

Family studies Xg^a and 12E7

RH BARC

MNS He

Enzyme treatment of rbc

Ge on erythroid cells

MER2/CD151

MNS ERIK

CD82 a 'new' tetraspanin on rbc

Mab - Wr^a

RhD Ψ gene

Mutations in EKLF/KLF1

RH splice site mutations

MNS MiIX

McLeod

Tn

Lutheran domain mutants

LFA JFV

Kell Ser193

MNS SAT

Miltenberger

Kell HDN

RH JAHK r^G molecular basis and serology

DI Sw^a

Lu(a-b-) family's in South Wales

Erythroid cell culture

Genetics and linkage

MNS Os^a & Ny^a

MNS Dantu

LW system

Immunoblotting : detection of glycophorin B with anti-S and anti-s

Proteome of adult and cord cells

LFA Jones

Red cell antigens during erythropoiesis

BEL-A2 erythroid cell line

DANE+ GYP(A-B-A) hybrid gene

VS and V blood groups in Africans

Blood group investigations – a Sherlock Holmes approach

- Patient with antibody
- Serological investigation - antibody, antigen
- Have we seen it before- is it different?
- Enzyme treatment of RBC
- SDS gel and immunoblotting
- Sanger sequencing



Results

Data analysis

New blood group/antigen? - Apply to ISBT for number

The example is going to illustrate how utilising different methods helped to solve a mystery

An obscure antigen achieves importance

- A red cell polymorphism serologically defined by two monoclonal antibodies (1987) named MER2
- The antigen was variable in strength
- Not given antigen or blood group status as no alloantibody of same specificity described
- None of the 8% of apparent MER2 negative people had made anti-MER2
- The gene encoding MER2 predicted to be located to chromosome 11p15

MER2.....

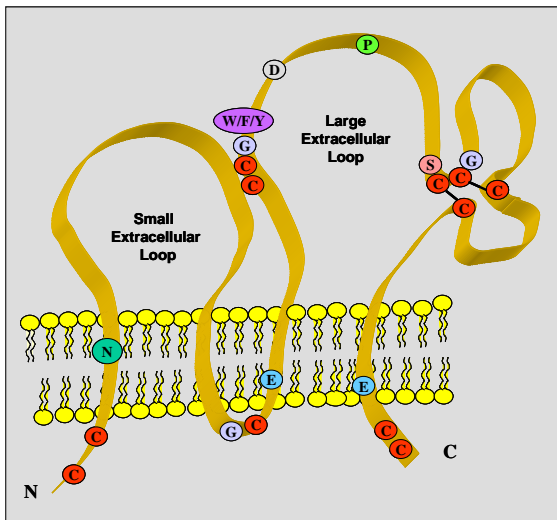
- Shortly after in 1988 allo anti-MER2 was identified in three (two related) individuals of Indian Jewish background
- In 1999 MER2 was given system status by the ISBT (RAPH)
- These MER2-negative individuals had devastating systemic illness, including kidney failure, sensorineural deafness, and epidermolysis bullosa
- In 2004 Dave Anstee noted that the gene location of a tetraspanin molecule, CD151, was the same of *MER2*



Are MER2 and CD151 related?
Problem CD151 reported not to be on rbc!

Tetraspanin CD151

- CD151 is a member of tetraspanin superfamily of proteins
- Tetraspanins first described in 1990's
- Very new to scientific research
- Function at time unclear, now known to have a role in cell adhesion, proliferation, signalling



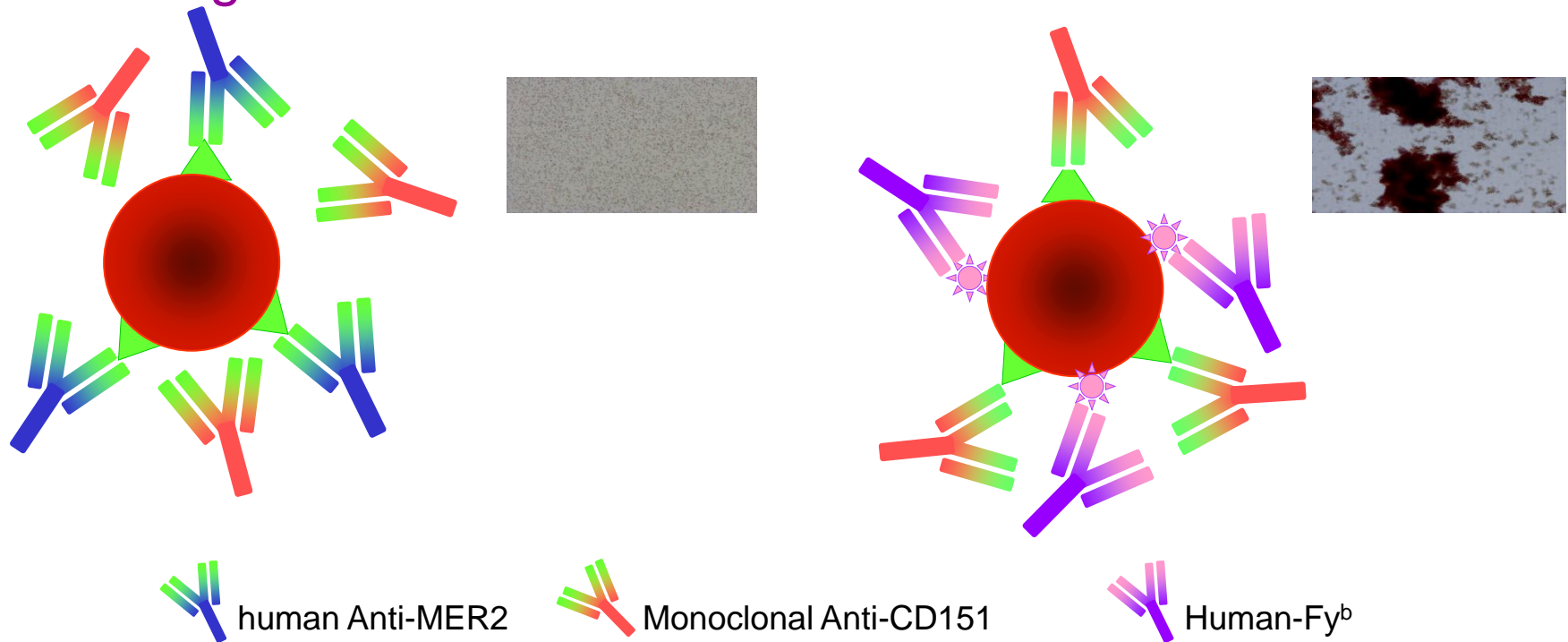
Tetraspanin molecule characterised by four hydrophobic domains and two extracellular loops

The Evidence

- Serological tests with anti-CD151 show it is on rbc
- Anti-CD151 and anti-MER2 react in a similar way
- Antigen strength in serological tests identical
- RBC from a 'true' MER2 negative individual was CD151 negative
- Are they detecting an antigen on the same protein?

Blocking studies

Does binding of human anti-MER2 to red cells block binding of anti-CD151?



Anti-MER2 blocked binding of anti-CD151
Evidence that CD151 and MER2 are on the same molecule

What we did next was easy (relatively!)

- Direct sequencing of all CD151 exons from the Israeli patients revealed a homozygous single nucleotide insertion G383 in exon 5
- This introduced a frameshift after Lys127 and a premature stop signal at codon 140
- The nucleotide insertion would result in a translated protein lacking most of the large extracellular loop (EC2) between transmembrane domains 3 and 4

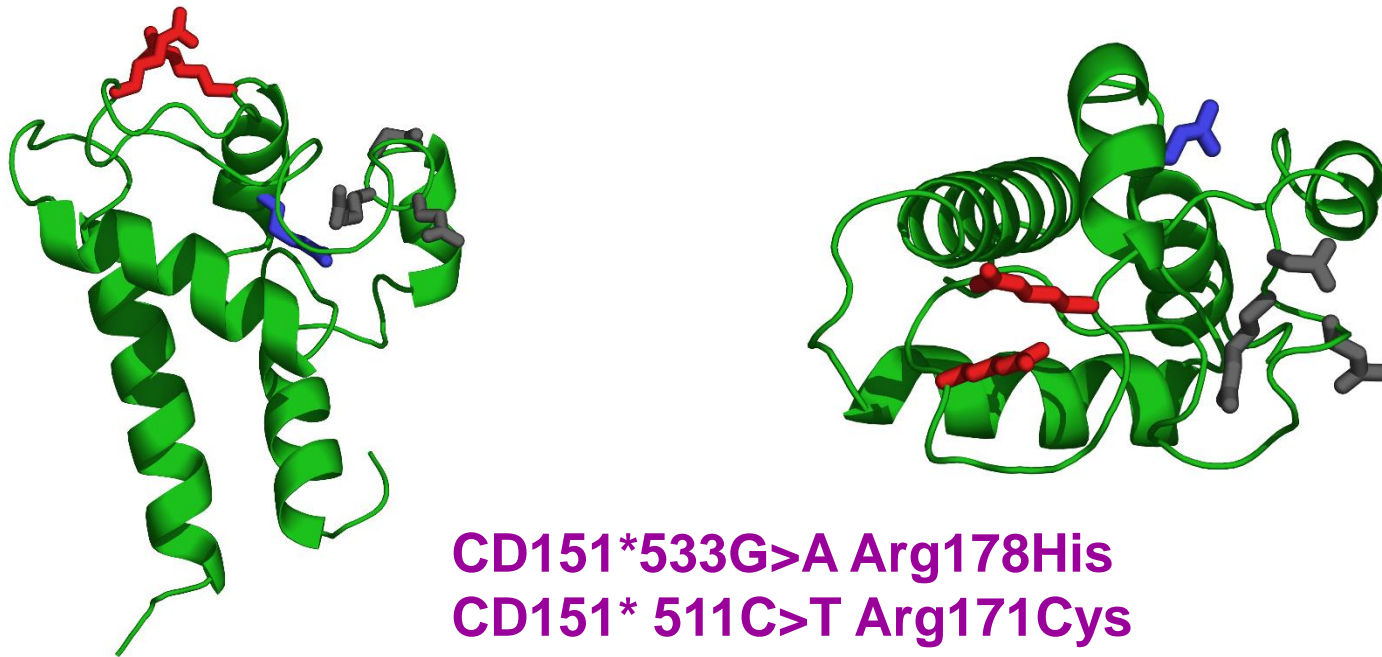
Protein unlikely to be inserted into membrane

MER2 neg probands identified by anti-MER2 in their plasma

- 3 Israeli Jews – ‘**natural gene knock outs**’ absence of CD151 protein with devastating biological effect
 - Healthy Turkish blood donor no transfusions
2 pregnancy’s **CD151*533G>A Arg178His**
 - Pakistani patient - 2 pregnancy’s
 - Turkish patient - transfusion and pregnancy
- } **CD151* 511C>T**
Arg171Cys

Molecular model CD151 with amino acid substitutions

Serology has also helped giving insights into visualisation of proteins and protein modelling



No significant structural rearrangement in CD151 protein

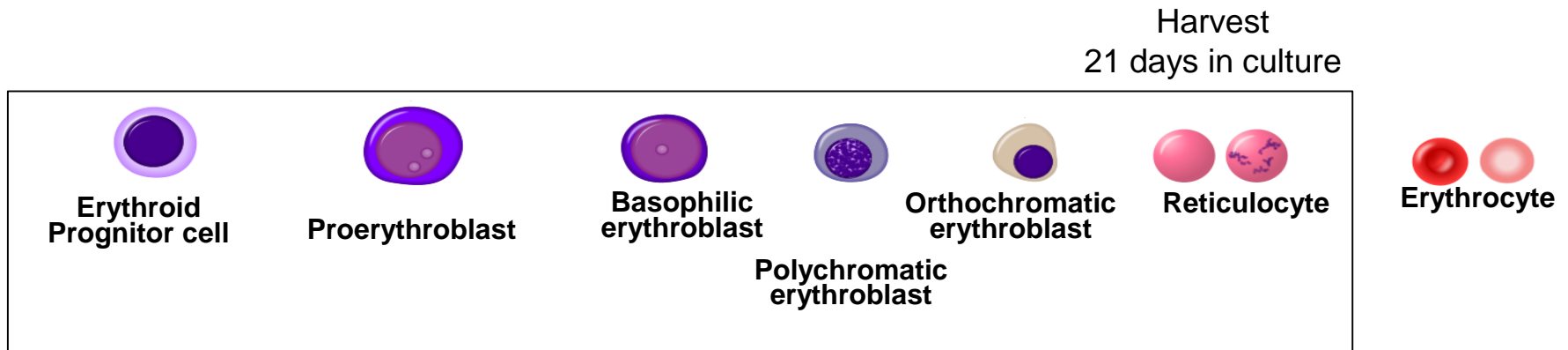
The slide features several red blood cells as a background. There are nine cells in total: five are perfectly circular and located in the upper half of the slide, while four are in the lower half. One cell in the lower-left area is tilted at an angle, while the others are oriented horizontally.

Ex vivo culture of erythroid cells

There are many novel powerful methods aiding serological investigations

Ex Vivo culture of erythroid cells

- Study of erythroid cells during culture
- Aspects of erythropoiesis: differentiation, morphology, size, viability, cell cycle, gene activity and apoptosis
- Surface antigens and order of expression
- GPC, Kell, RhAG, GPA, Band 3, Rh, GPB, Duffy, Lutheran



cRBC as a tool for investigating Rh anomalies

- Sequencing genomic DNA often fails to explain cause of serologically altered expression of Rh antigens
- Degraded DNA from similar molecules (RhAG, RhD, RhCE, RhBG, RhCG) present in plasma at cell death
- cDNA produced from whole blood RNA unsuitable

Could cDNA produced from RNA isolated from cRBCs avoid this problem?

Variant expression of E antigen

- Sample referred from Switzerland/Germany (P Bugert, H Hustinx)
- Red cells of a regular blood donor (group A DccEe R₁R₂) failed to react with 4/14 monoclonal anti-E
- Sequence of gDNA revealed a heterozygous c.939G>A (Pro313) at the last nucleotide of exon 5 of *RHCE* – in the splice site
- This mutation failed to explain the aberrant E antigen

Did the 939G>A mutation influence the splicing of *RHCE*?

loss of part or whole exon / inclusion of a part or whole intron
frame shifts / premature stop codon / truncated or altered proteins

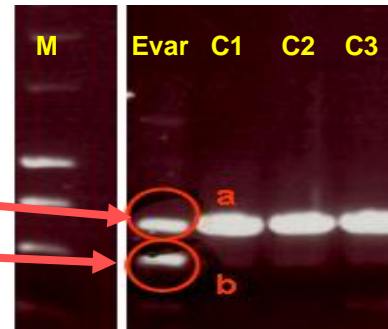
PCR amplification and sequencing *cRHCE*

- 1) Erythroid cell culture to obtain mRNA
- 2) PCR amplification

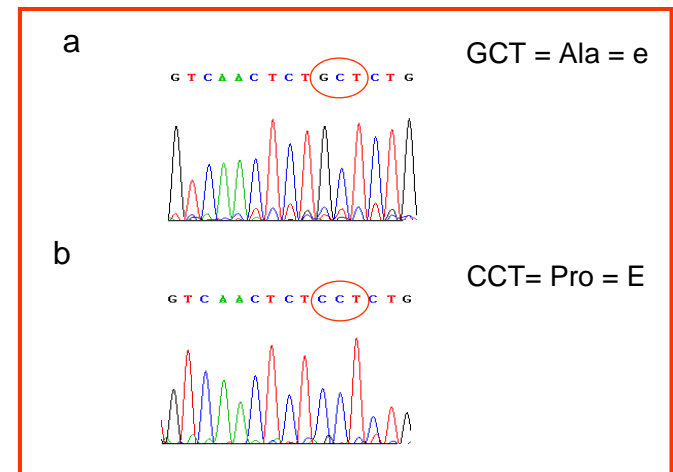
Two cDNA products observed:

Band a - full length *RHCE*

Band b - truncated *RHCE*

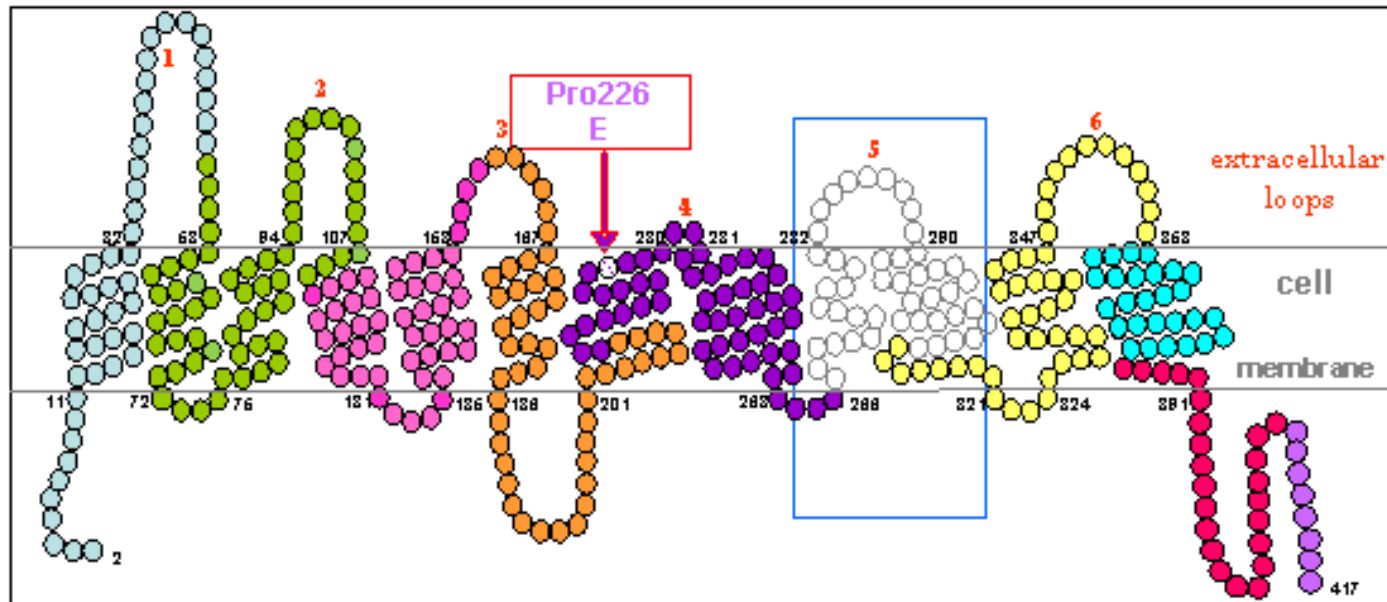


- a) wild-type cDNA has 676G Ala226 = e antigen
- b) Truncated cDNA has 676C Pro226=E antigen
lacks exon 6 but remains in frame



RhCE protein model

‘truncated’ CE protein



- Almost complete deletion of transmembrane regions 9,10 and the 5th extracellular loop
- Loss of 46 amino acids

cDNA from cultured cells successful

Ex vivo culture of erythroid cells



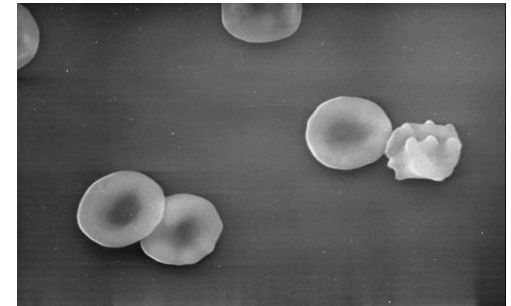
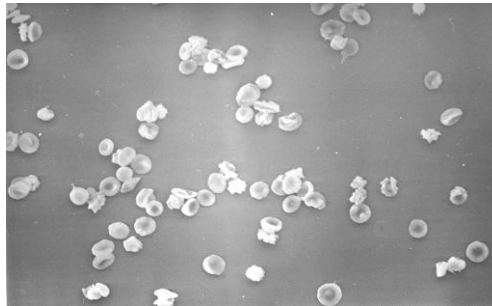
- Blood and Transplant Research Unit (BTRU)
- Ultimate aim to produce a clinical product which will improve transfusion safety
- Initially to provide blood for multi-transfused patients e.g. sickle cell disease or those with rare groups
- May provide 'designer' cells for control panels by gene manipulation

RESTORE (Recovery and Survival of Stem Cell
Originated Red Cells)

Assessment of *ex-vivo* produced RBCs

SEM Cultured reticulocytes

Cells appear normal in shape,
7-8 μ m in size



- Safety and function
- Morphology
- Microbiological testing
- Oxygen carrying capacity
- Deformability
- Viability on storage
- **SEROLOGY**

Serological evaluation of cRBC

- Cell size: cRBCs are late reticulocytes - larger than mature RBC
 - How do they perform in standard serological tests?
 - Can they be tested using gel card technology?
 - Is antigen expression the same as on native rbc
 - Are cells polyagglutinable
 - Expression of Neo antigens?
-
- ❖ Guidelines for compatibility testing must be followed
 - ❖ Suitability and reliability of method is important!

Phenotype of cRBC v native RBC

CRBC from peripheral blood of an adult

		ABO	M	N	S	s	P ₁	Rh	Lu ^a	Lu ^b	K	k	Kp ^a	Le ^a	Le ^b	Fy ^a	Fy ^b	Jk ^a	Jk
adult	rbc	O	+	+	-	+	-	R ₁ r	-	+	-	+	+	-	+	-	+	-	+
	cRBC	O	+	+	-	+	+++	R ₁ r	-	+	-	+	+	-	+	-	+	-	+

Good antigen expression of all major blood group antigens

However, discrepant results for P₁ antigen

Carbohydrate antigens cRBC v native RBC

Anti-A (titration scores)

Anti-A	Native rbc	mRBC
Adult 1	98	59
Adult 2	94	56
Group A ₁ control	88	
Group A ₂ control	66	

Dolichos biflorus

Dolichos biflorus	Native rbc	mRBC
Adult 1	C	0
Adult 2	C	0
Group A ₁ control	C	
Group A ₂ control	neg	

cRBC from two adults show reduced A antigen and group as A₂ their mature erythrocytes are A₁

Anti-P₁ and antibodies to globoside antigens

	Anti-P ₁	Anti-P	Anti-P ^k	Anti-LKE
Adult RBC	0	+++	0	++++
cRBC	++++	+++	++	0

Disruption of P₁ and globoside antigens

Carbohydrate antigens have altered expression on cRBC
Secondary gene products – glycosylation incomplete?

Possible to use serology as a marker of maturity?

Testing cells from 5 erythroid cultures

80 ABO compatible 'fresh' donor plasma tested with cRBCs

LISS IAT tube tests

LISS IAT gel cards

NO UNTOWARD REACTIONS OBSERVED

cRBCs test with Lectin panel; *Arachis hypogea*, *Ulex europeus*,
Glycine soja

NO POLYAGGLUTINATION DETECTED

NO NEO ANTIGENS DETECTED

Dantu Revisited 2017

Serology and Genetics of an MNSs-Associated Antigen Dantu

Contreras M, Green C, Humphreys J, Tippet P, Daniels G, Teesdale P, Armitage S, Lubenko A

Vox. Sang. Jun 1984 46; 6 341-423

Resistance to malaria through structural variation of red blood cell invasion receptors

Leffler EM, Band G, Busby GBJ, Kivinen K, Le QS, Clarke GM, Bojang KA, Conway DJ, Jallow M, Sisay-Joof F, Bougouma EC, Mangano VD, Modiano D, Sirima SB, Achidi E, Apinjoh TO, Marsh K, Ndila CM, Peshu N, Williams TN, Drakeley C, Manjurano A, Reyburn H, Riley E, Kachala D, Molyneux M, Nyirongo V, Taylor T, Thornton N, Tilley L, Grimsley S, Drury E, Stalker J, Cornelius V, Hubbart C, Jeffreys AE, Rowlands K, Rockett KA, Spencer CCA and Kwiatkowski DP

Science. 2017 Jun 16;356(6343)

Dantu MNS variant

- Serological evidence that Dantu is part of MNS blood group system
- Dantu is associated with very weak s antigen, protease resistant N antigen and very weak or absent U antigen
- Three types of Dantu recognised
- GP(B-A) hybrid molecule

Dantu and invasion of RBC by *P. falciparum*

- Genome sequence of 3269 individuals from sub-Saharan Africa
- Identified varied copy number variants affecting the host invasion receptor genes *GYPA* and *GYPB*
- Dantu reduces the risk of severe malaria by 40%
- Dantu has recently risen in frequency in parts of Kenya
- These findings link structural variation of red blood cell glycoprotein with natural resistance to severe malarial invasion

Importance of Serology in Biology

- Role of transferases -study of carbohydrate antigens
- Rh and MNS systems, recombination between closely linked homologous genes resulting hybrid proteins
- Kell an endopeptidase –vasoconstrictor
- Xk and McLeod syndrome
- Cromer and DAF (CD55) (decay accelerating factor)
- CD59 PNH
- Colton and AQP1 water channels
- Genetics – autosomal, X-linkage, X-inactivation,
- CD99 antigen on both X and Y chromosome
- Tetraspanins
- Rh, RhAG, Diego (Band 3), LW, GPA, GPB, CD47 complex
- Dantu receptor for malaria

Does serology have a role in 2017?

- Plays an important role in identifying rare and null phenotypes
- Makes valuable contributions to genetics, cellular interactions and the function of blood group antigens and their carrier molecules
- Blood is an easily available tissue and many molecules on RBC also present on other tissues

It remains a requirement of the International Society of Blood Transfusion red cell nomenclature committee. who administer and organise new blood group antigen that they be defined serologically by a specific alloantibody

Ruth Sanger and Rob Race



Patricia Tippet & Geoff Daniels



Dave Anstee



Louise Tilley & Vanja Crew

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Tosti Mankelow

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