



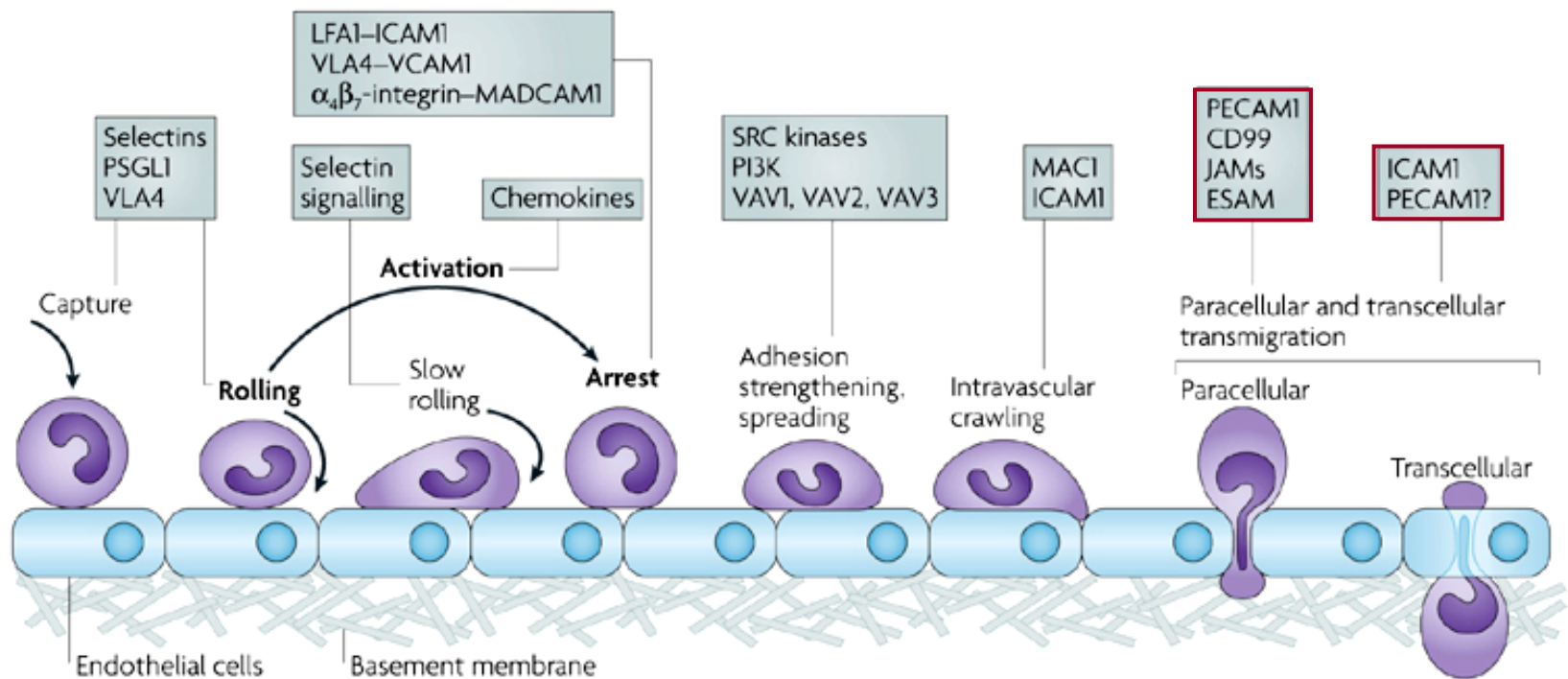
Neutrophils: Immunohematology - Innate Immunity

Dr. Sentot Santoso PhD

Conference
Safety on Platelet Transfusion
Guangzhou, PR China
27-30 April 2009

Getting to the Site of Inflammation

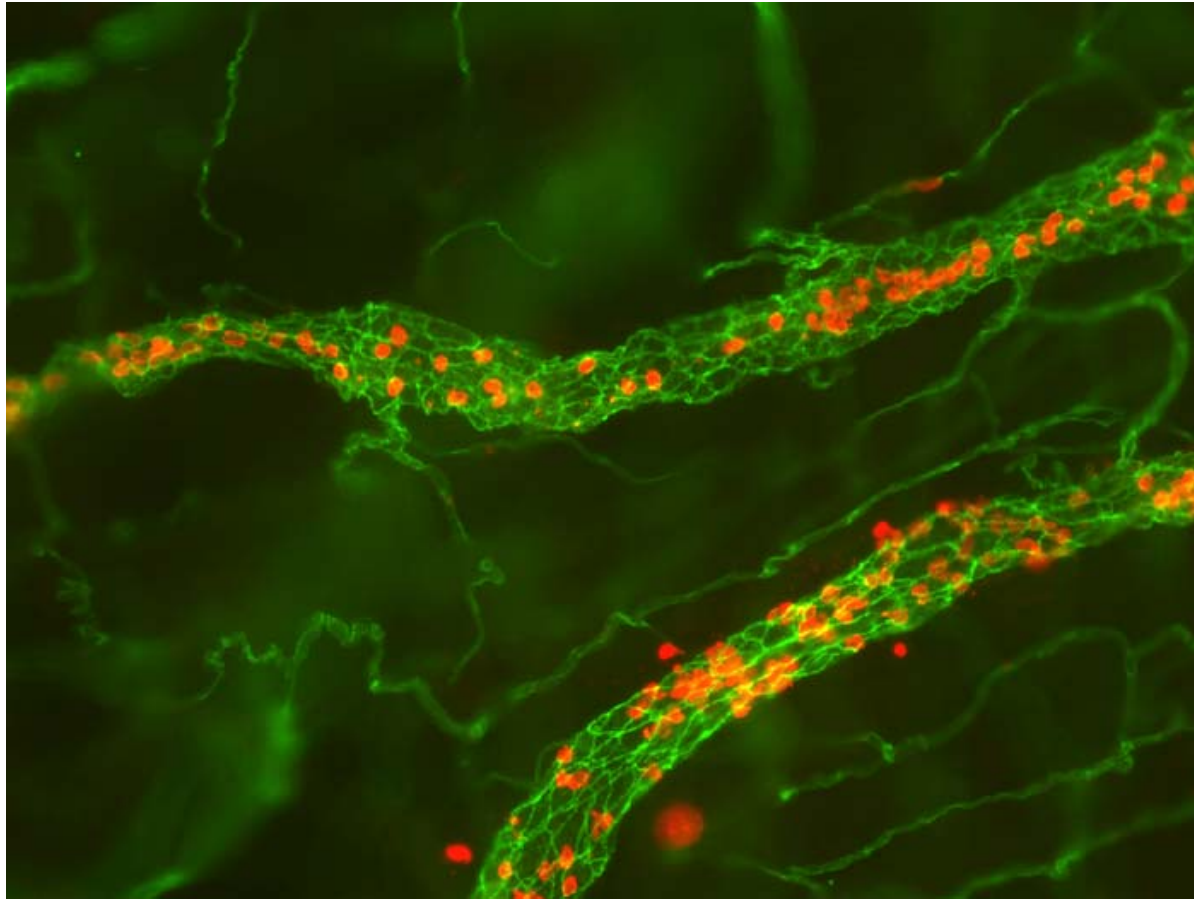
Leukocyte Adhesion Cascade



site of inflammation

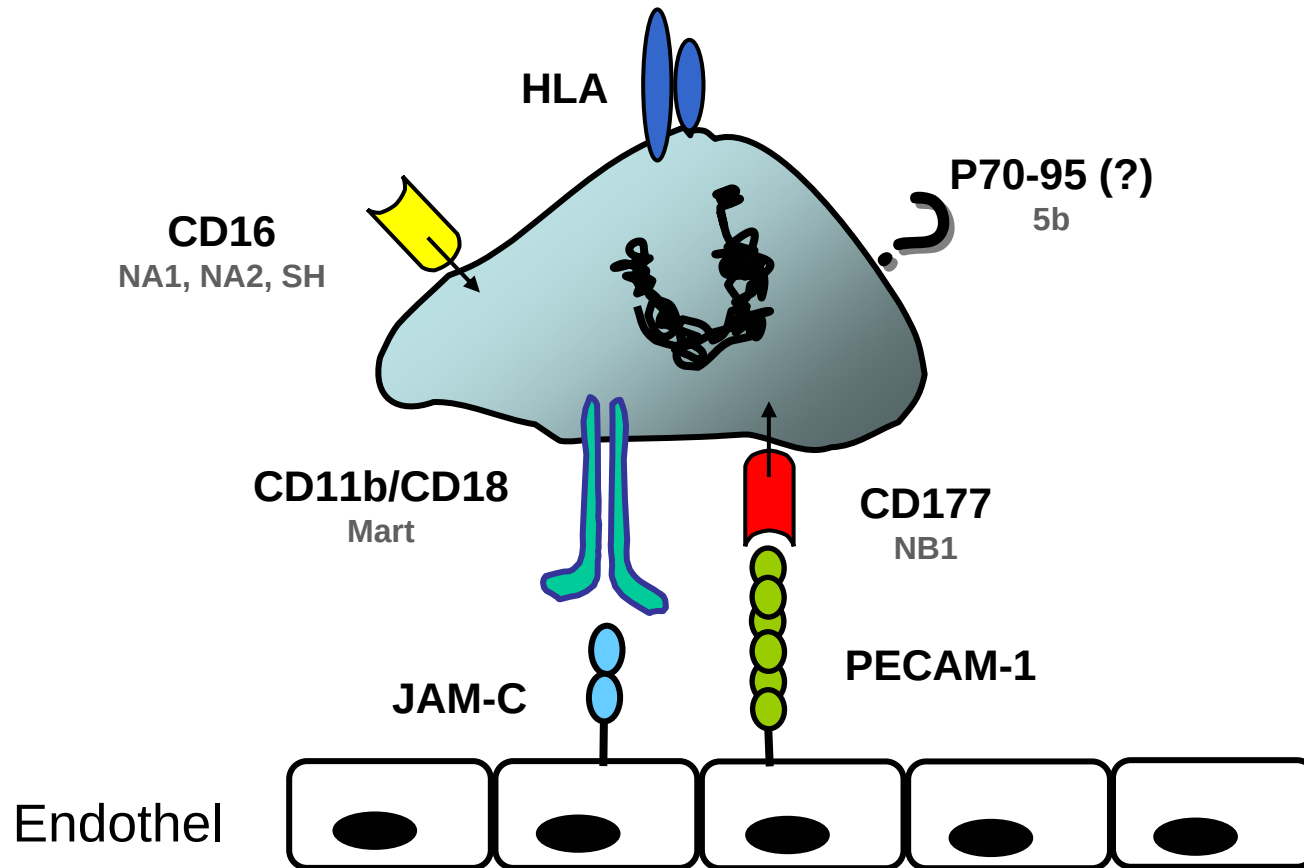
Getting to the Site of Inflammation

Leukocyte Adhesion Cascade

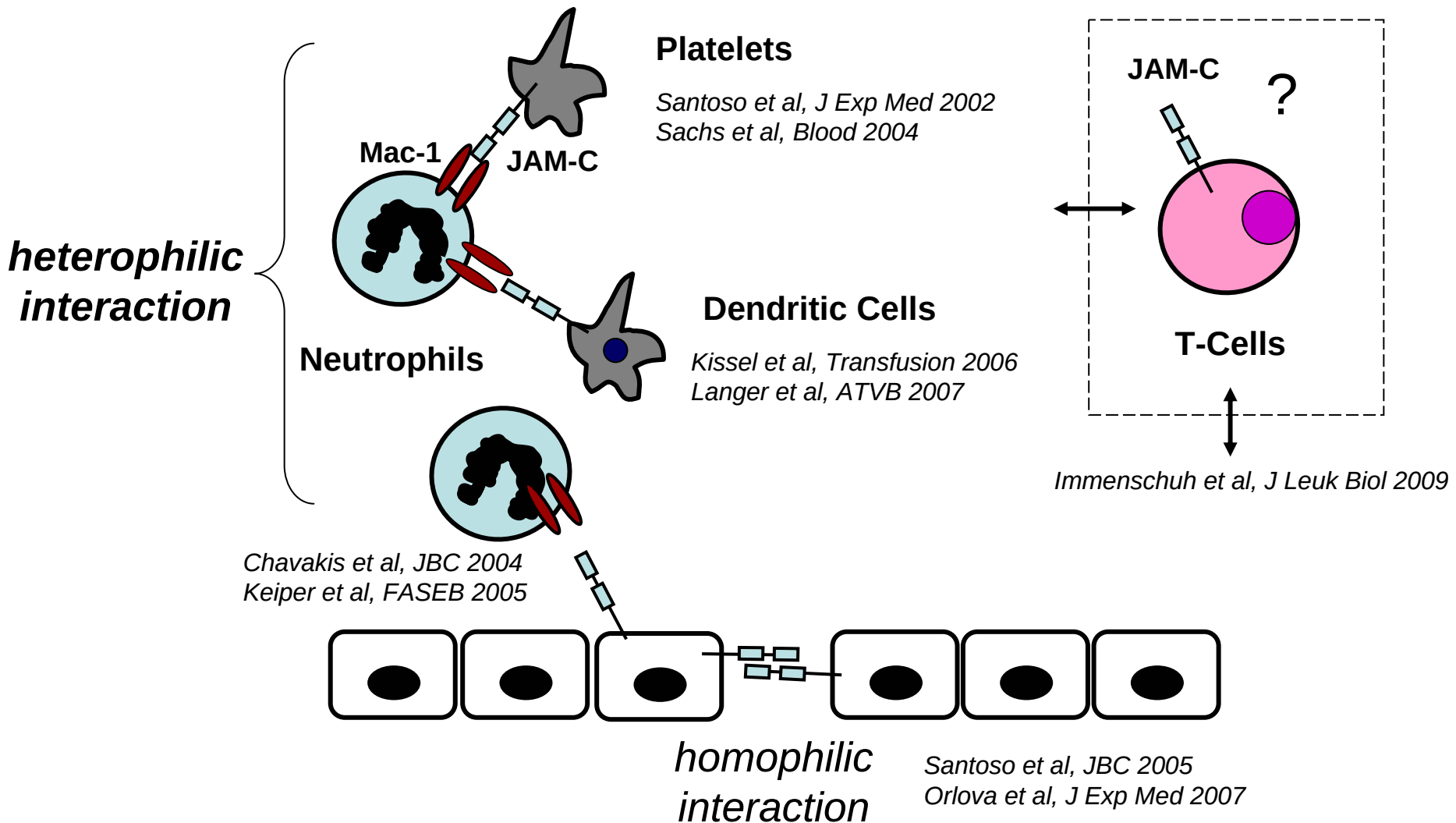


Courtesy of Dr. D. Vestweber, Münster

Human Neutrophil Antigen (HNA)



JAM-C mediated cross-talk between different cells

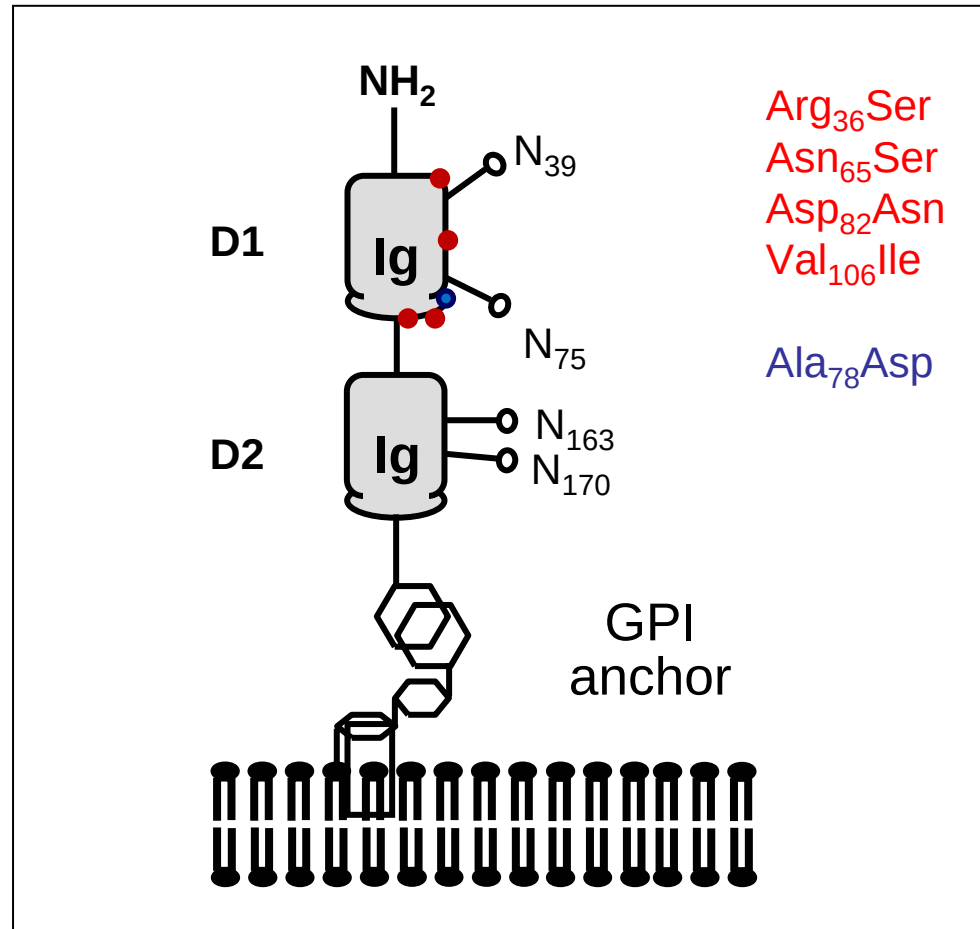


Human Neutrophil Antigen (HNA)

Phenotyp	HNA	Localisation	Frequency Whites	Frequency Asians*
NA1	1a	Fc γ RIIIb	57-62%	88-91%
NA2	1b	Fc γ RIIIb	88-89%	51-54%
SH	1c	Fc γ RIIIb	5%	<1%
	HNA-1 null		0.15%	<1%
NB1	2a	CD177	89-99%	98%
5b	3a	<i>not known</i>	89-96%	?
Mart	4a	CD11b	99%	>99%
Ond	5a	CD11a	86-92%	81%

* Chinese, Japanese, Koreans, or Taiwanese

HNA-1 on Fc γ RIIb (CD16b)



NA2

SH

● point mutation
—○ oligosaccharide

Clinical Impact of Neutrophil Antibodies

Febrile non-haemolytic transfusion reactions

Transfusion-related acute lung injury (TRALI)

Neonatal alloimmune neutropenia

Autoimmune neutropenia, and

Post-bone marrow transplant neutropenia

Laboratory Diagnostic of Neutrophil Antibodies

Granulocyte Immunofluorescence Test (GIFT)

Granulocyte Agglutination Test (GAT)

Lymphocyte Immunofluorescence Test (LIFT)

Monoclonal Antibody Immobilized Granulocyte Antigens (MAIGA)

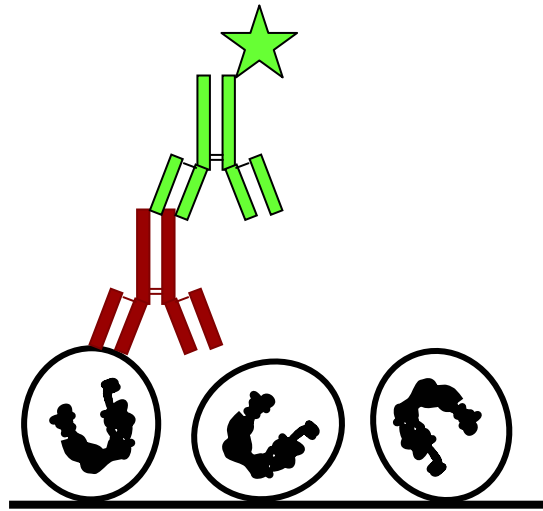
ELISA

Neutrophil Alloantibodies

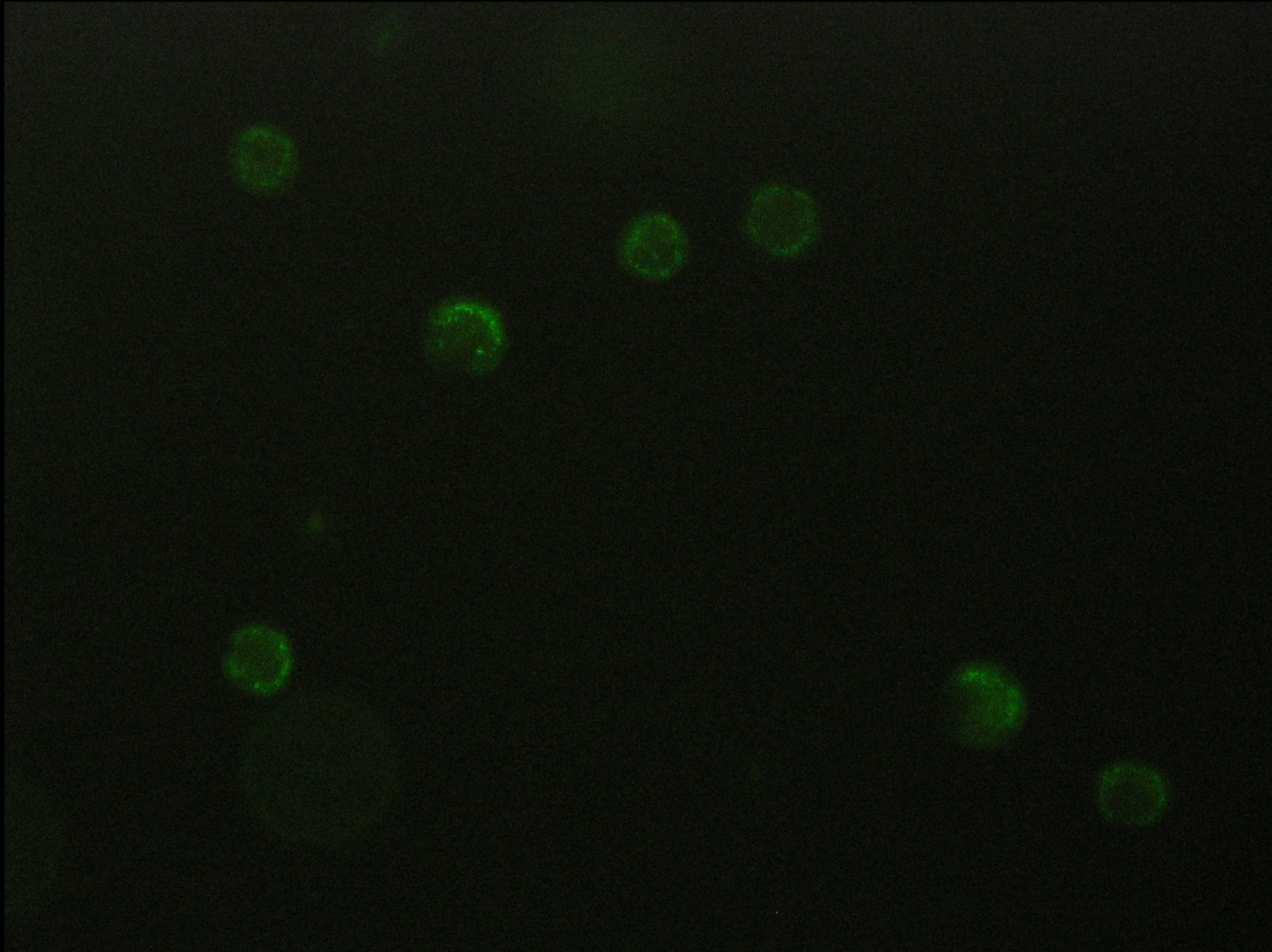
found in our laboratory (n=31)

NA1	10	HNA-1a	}	75%
NA2	8	HNA-1b		
SH	0	HNA-1c		
ND1	6	HNA-1		
NB1	4	HNA-2a	}	25%
5b	1	HNA-3a		
Mart	2	HNA-4a		
Ond	0	HNA-5a		

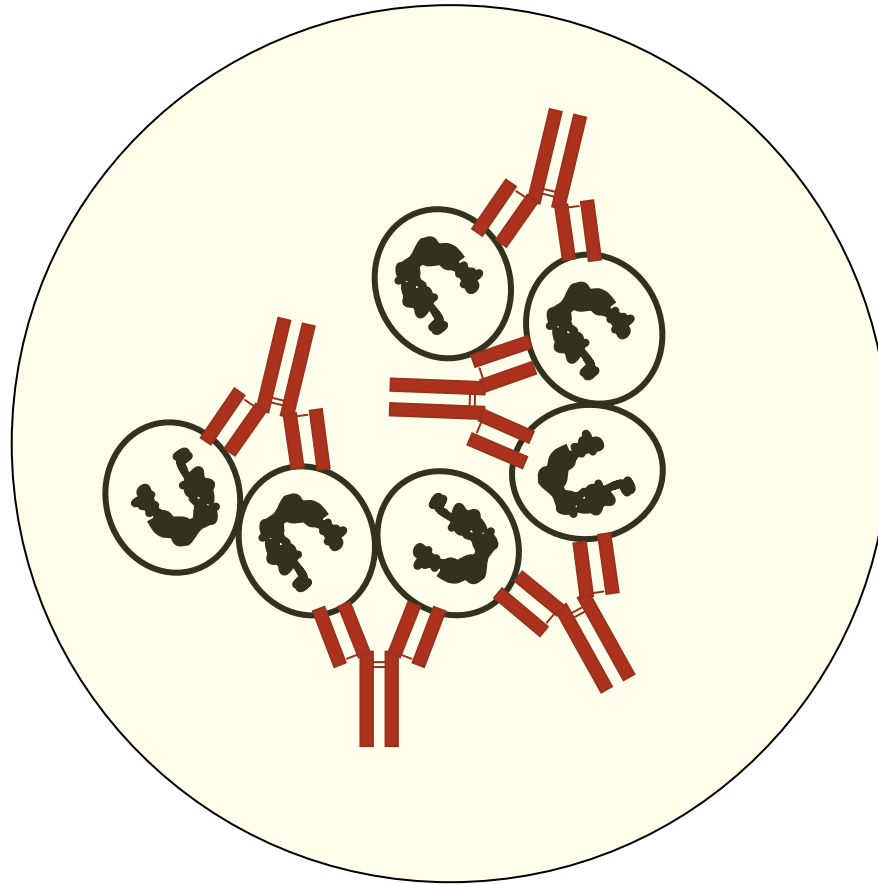
Granulocyte Immunofluorescence Test (GIFT)



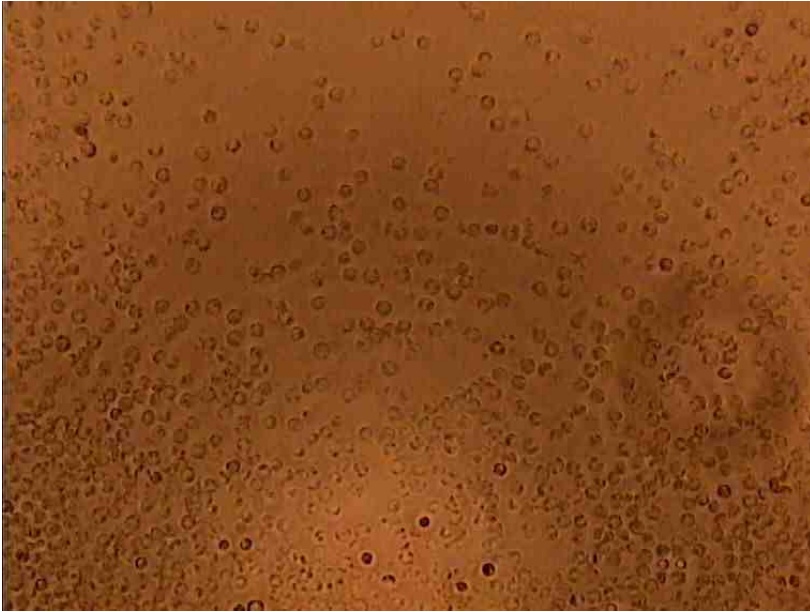
Granulocyte Immunofluorescence Test (GIFT)



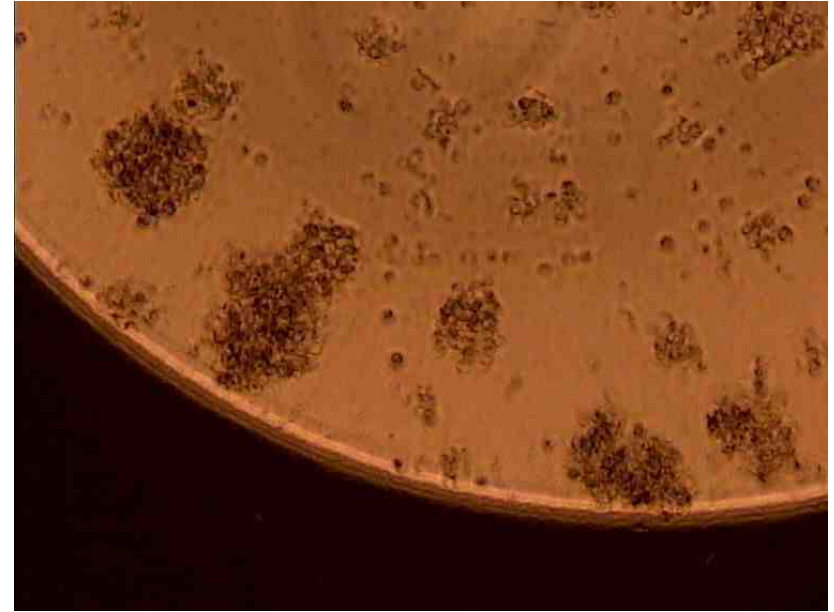
Granulocyte Agglutination Test (GAT)



Granulocyte Agglutination Test (GAT)

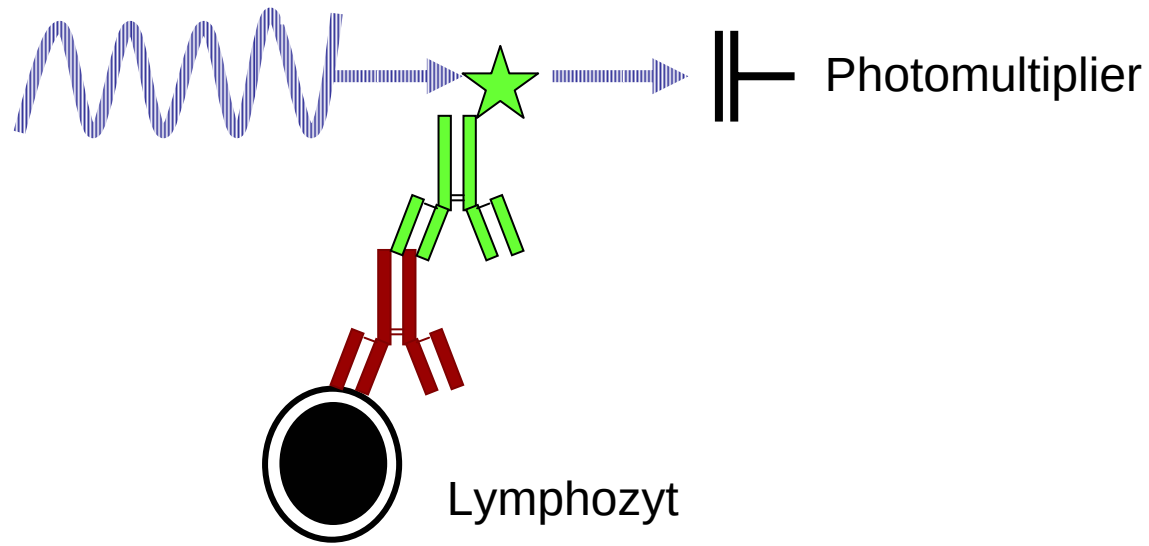


GAT = 0
(Inkubation mit AB-Plasma)

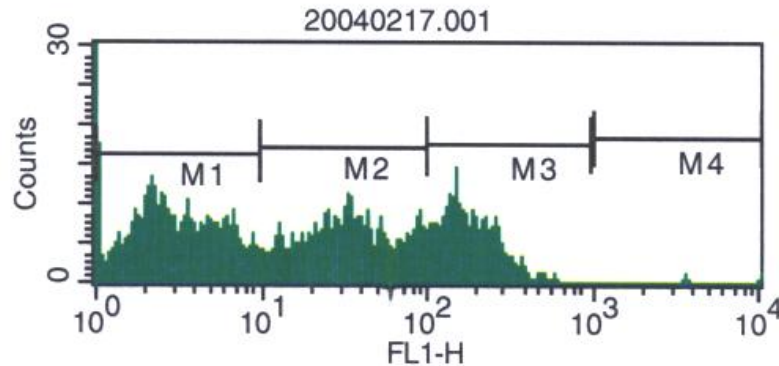


GAT = 80
(Inkubation mit Anti-HNA-3a)

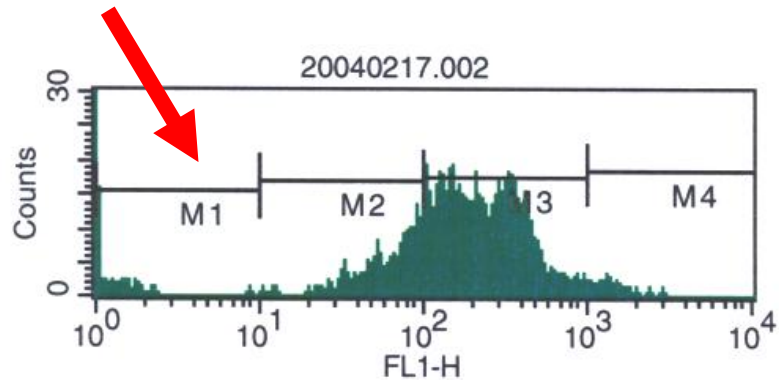
Lymphocyte Immunofluorescence Test (LIFT)



Lymphocyte Immunofluorescence Test (LIFT)



- AB-Plasma



- Anti-HLA-A2

Serology Pattern (an example)

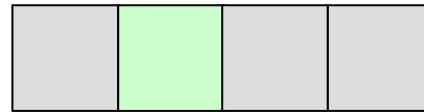
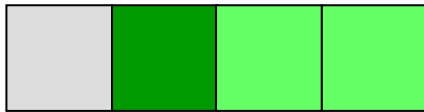
GIFT

GAT

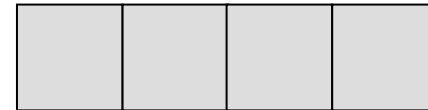
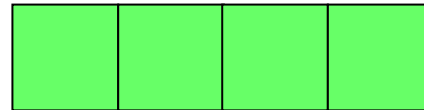
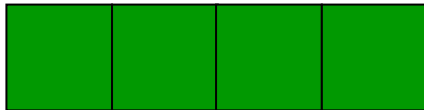
LIFT



HLA



NA2

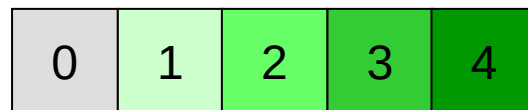


Auto-Ab



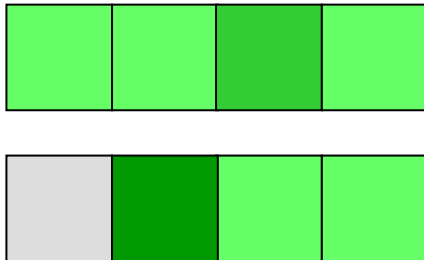
negative

Reactions 0-4:

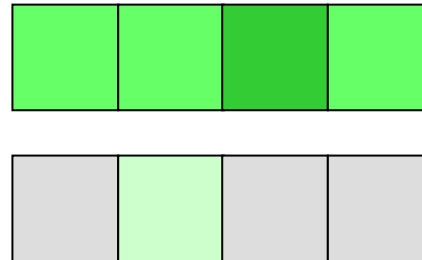


Problem with HLA-Immunization

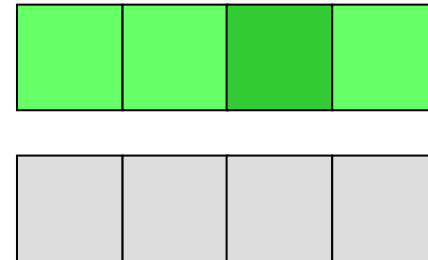
GIFT



GAT



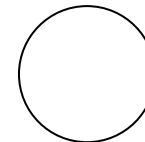
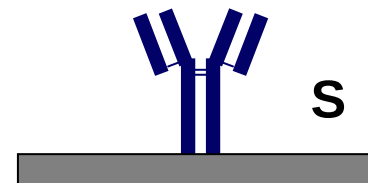
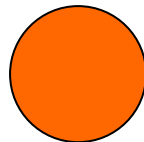
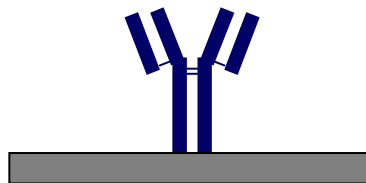
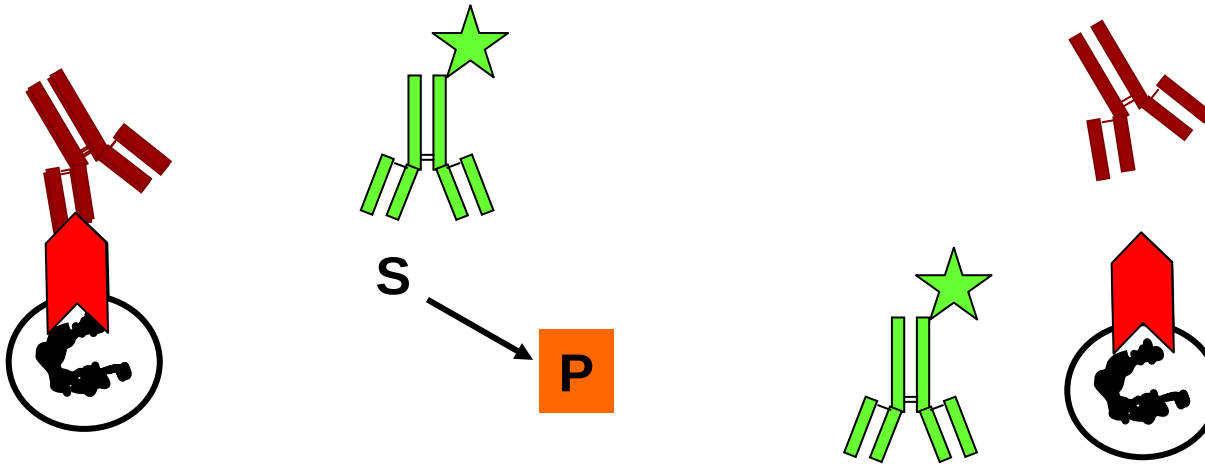
LIFT



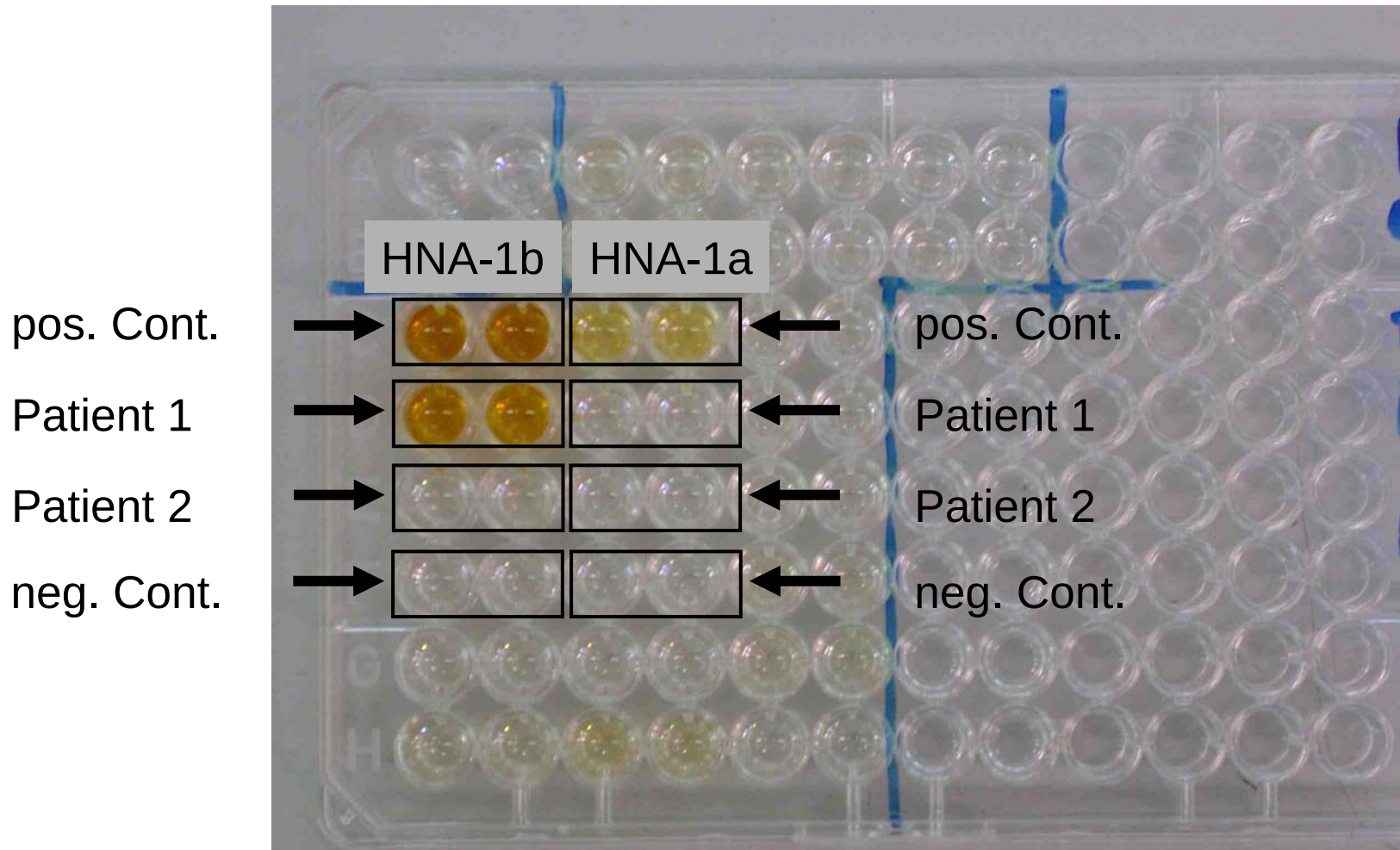
HLA

?? NA2

MAIGA



MAIGA



Patient 1: Anti-NA2; Patient 2: negativ

Advantages and Disadvantages of Antigen Capture Assays

Advantages	Disadvantages
highly specific	washing steps
wide spectrum of GPs	need of mabs
robust	need of fresh granulocytes
objective	anti mouse
standardized	time consuming
accepted	experienced technologist

Simplify Granulocyte Serology by ELISA

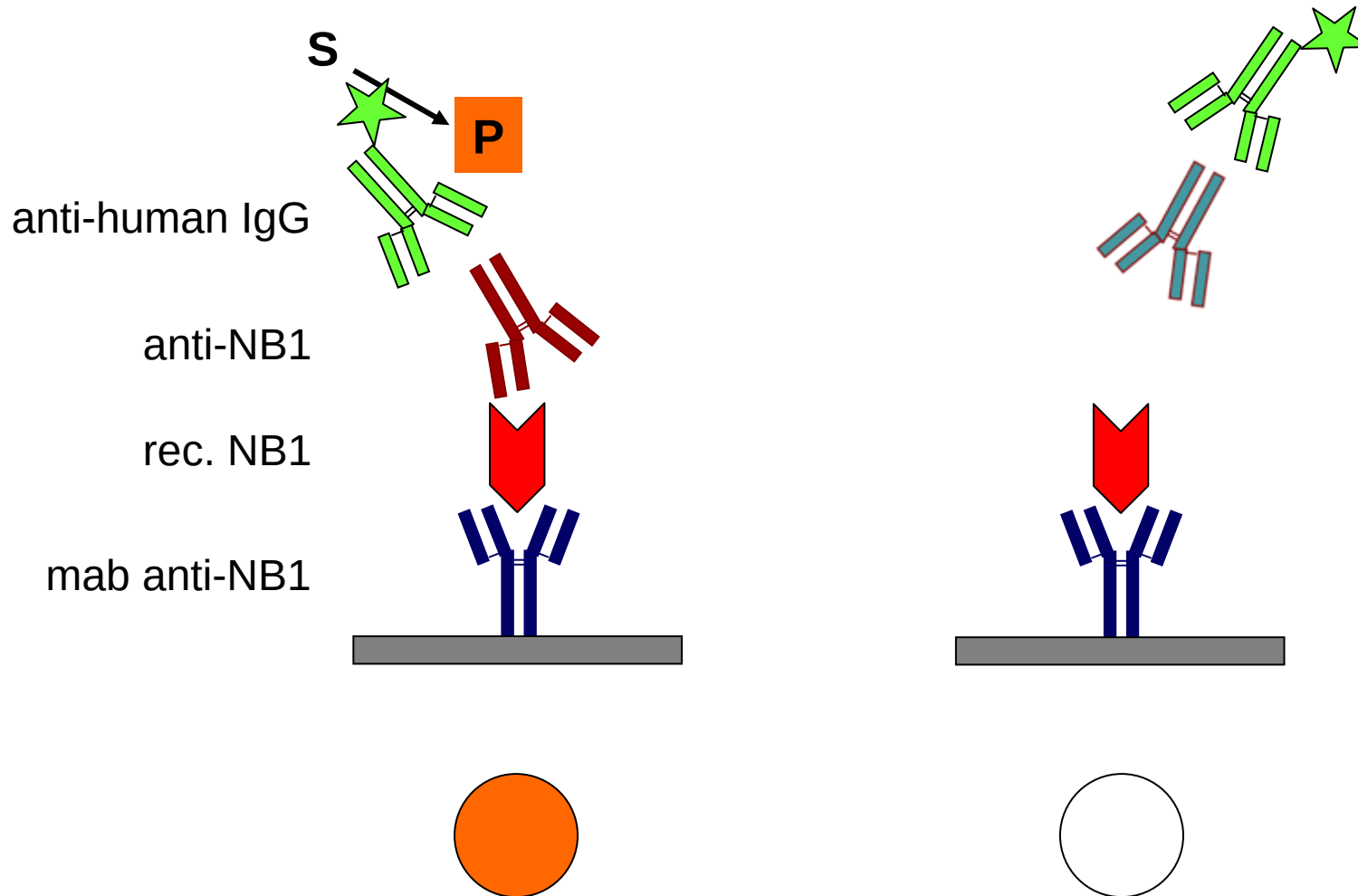
A novel ELISA method for the detection of HNA-2a neutrophil antibodies

Behnaz Bayat, Silke Werth, Ulrich J. H. Sachs and Sentot Santoso

Institute for Clinical Immunology and Transfusion Medicine,
Justus Liebig University, Giessen, Germany

Transfusion (in press)

Rapid ELISA



Rapid ELISA: First Result

No. of sera	Antibody Specificities	ELISA (OD ₄₅₀)	MAIGA (OD ₄₉₂)	
			undiluted serum	diluted serum 1:10
1	HNA-2a	0.72	0.57 (+)	2.30 (+)
2	HNA-2a	0.28	2.74 (+)	1.60 (+)
3	HNA-2a	0.81	0.53 (+)	2.05 (+)
4	HNA-2a	1.46	1.33 (+)	0.99 (+)
5	HNA-2a	0.53	2.14 (+)	2.25 (+)
6	HNA-2a	0.02	1.11 (+)	1.05 (+)
7	HNA-2a	0.44	2.66 (+)	2.24 (+)
9	HNA-2a	1.10	<u>0.39 (-)</u>	1.98 (+)
10	HNA-2a	0.98	0.71 (+)	1.14 (+)
11	Anti-PR3	0.06	0.59 (+)	0.47(+)
13	Anti-PR3	0.05	1.78 (+)	1.50 (+)
14	Anti-PR3	0.01	1.71 (+)	1.66 (+)
15	Anti-PR3	0.01	1.64 (+)	1.51 (+)
17	Anti-PR3	0.03	1.09 (+)	0.90 (+)
18	Anti-PR3	0.01	1.73 (+)	1.14 (+)

Genotyping Analysis

<i>Genotype</i>	<i>Phenotype</i>	<i>PCR</i>
HNA-1a	NA1	Bux et al., 1994
HNA-1b	NA2	
HNA-1c	SH	Bux et al., 1997
HNA-2a	NB1	_*
HNA-3a	5b	_**
HNA-4a	Mart	Sachs et al., 2004
HNA-5a	Ond	Sachs et al., 2005

* *multiple polymorphisms (Kissel et al., 2002)*

** *molecular genetic base not known*

HNA Genotyping Analysis



BAGene

HNA-TYPE

CE

LOT 0901 HN

IVD

2010-07

REF

6670

Worksheet und Auswertetabelle / Worksheet and Evaluation diagram

Reaktions-Nr. / Reaction No.	1	2	3	4	5	6	7
PCR-Produkt (Größe in bp) PCR product (size in bp)	141	219	191	249	249	283	283
Antigen Antigen	NA1	NA2	SH	Mart (positive)	Mart (negative)	Ond (positive)	Ond (negative)
HNA-Merkmale HNA specificities	HNA-1a	HNA-1b	HNA-1c	HNA-4a	HNA-4b	HNA-5a	HNA-5b
Beispiele / Examples							
HNA-1ab, 4ab, 5aa	+	+	-	+	+	+	-
HNA-1aa, 4aa, 5aa	+	-	-	+	-	+	-
HNA-1bc, 4aa, 5bb	-	+	+	-	-	-	+
HNA-Genotyp HNA genotype	1	2	3	4	5	6	7

Proben-ID / Sample-ID:

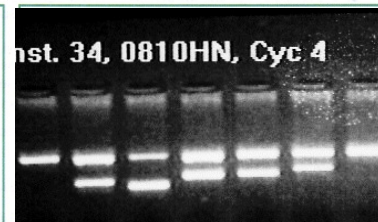
Probenname / Name:

Geb.-Datum / Birthdate:

Ergebnis / Result:

Datum / Date:

Unterschrift / Signature:



Clinical Impact of Neutrophil Antibodies

Febrile non-haemolytic transfusion reactions

Transfusion-related acute lung injury (TRALI)

Neonatal alloimmune neutropenia

Autoimmune neutropenia, and

Post-bone marrow transplant neutropenia

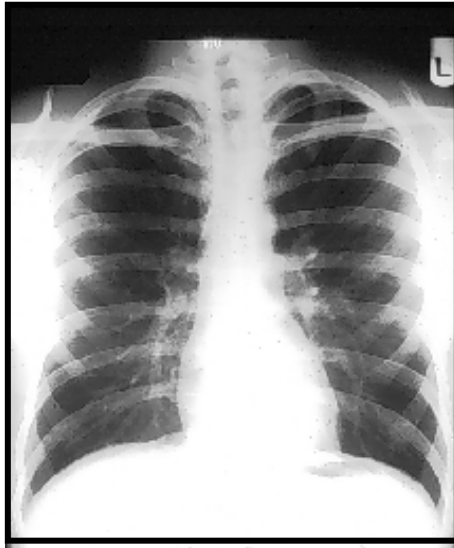
Transfusion Related Acute Lung Injury (TRALI)

- is an uncommon but potentially fatal reaction to transfusion of plasma-containing blood components (FFP, platelets)
- acute respiratory distress, non-cardiogenic lung oedema temporal association with transfusion and hypoxaemia
- lung oedema, capillary leukocytosis and neutrophil extravasation
- leukocyte antibodies in plasma and platelet concentrates or/and neutrophil priming agents in stored blood components

TRALI is the most common cause of transfusion related mortality

TRALI

**Before
Transfusion**

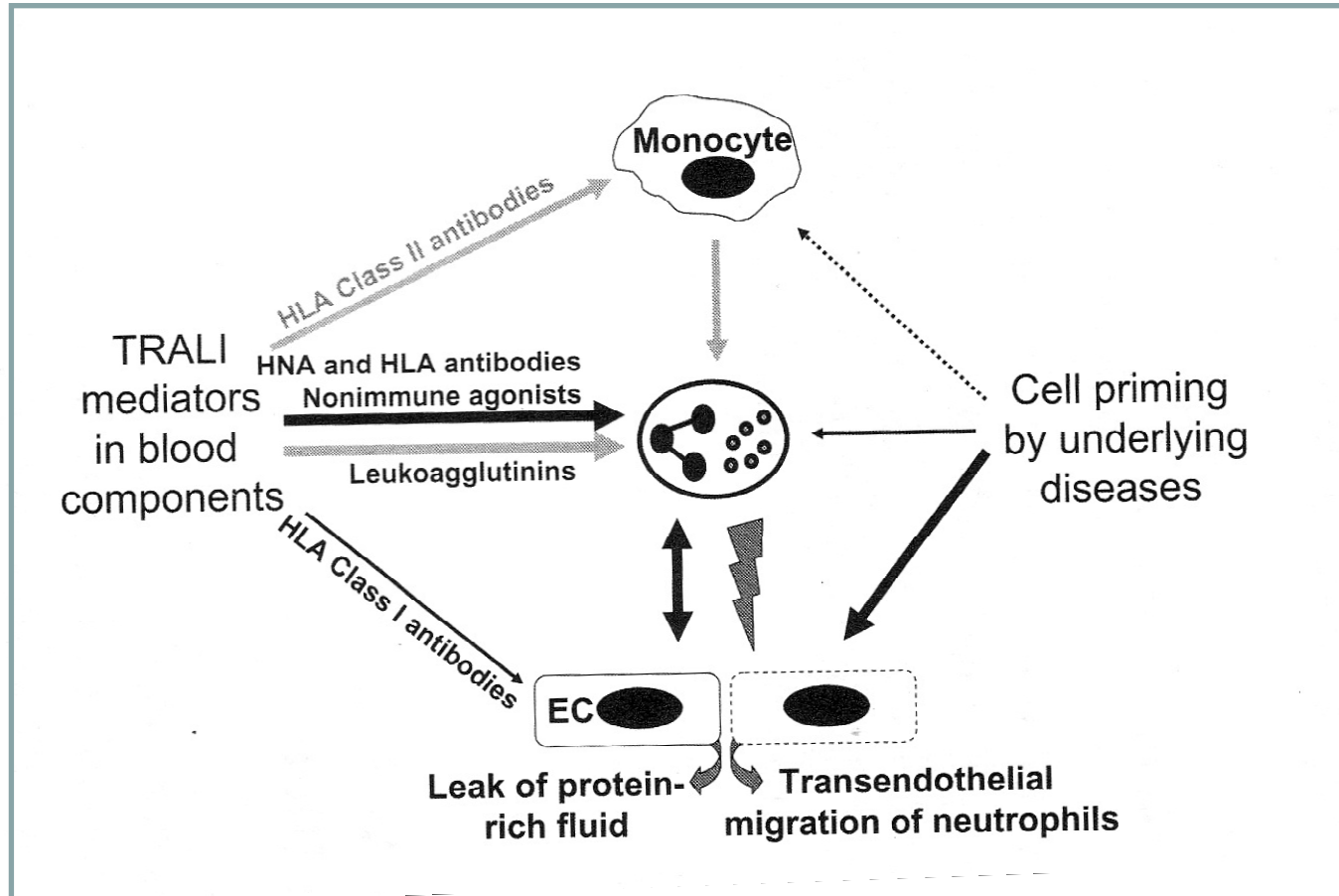


**4 h After
Transfusion**

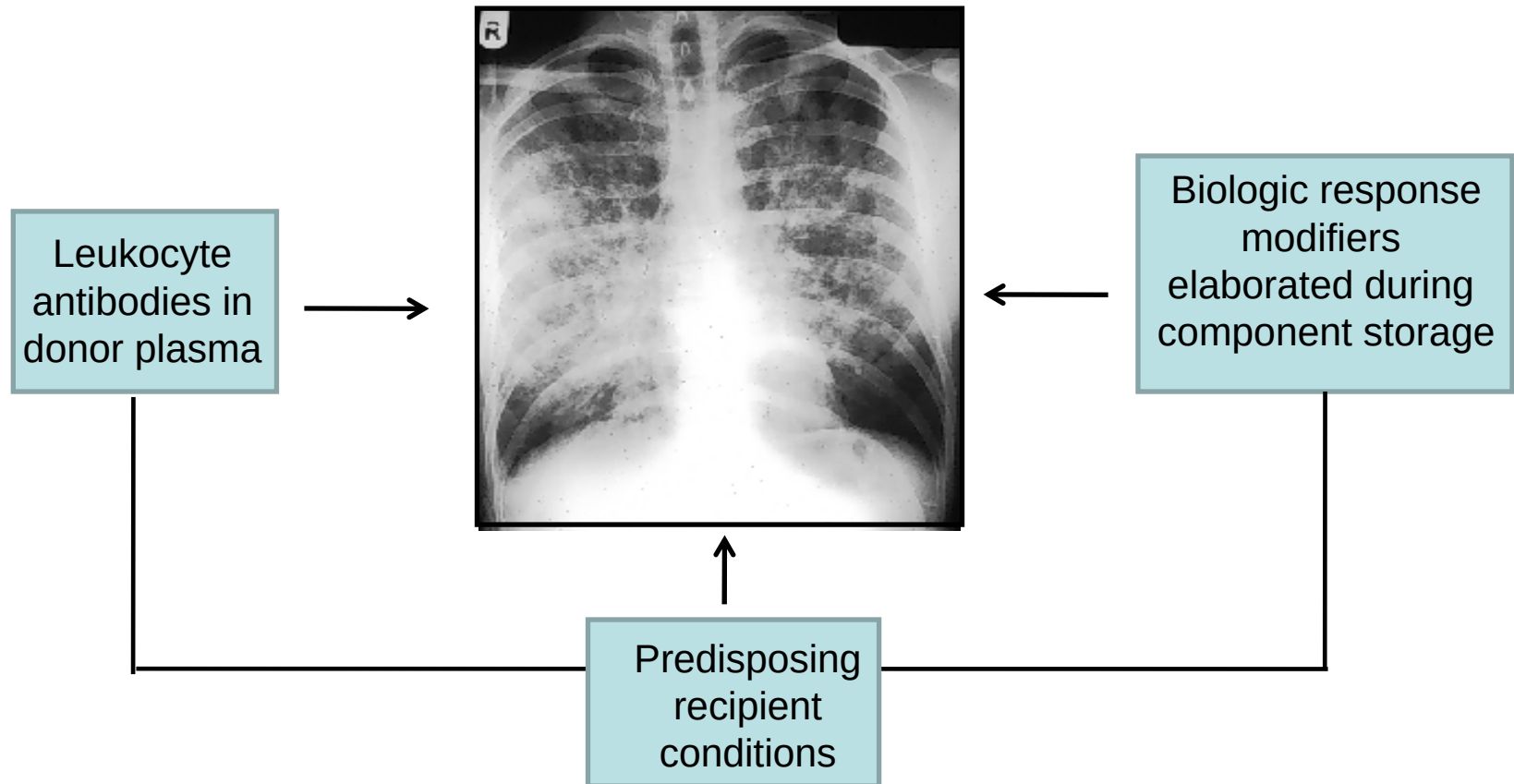


Courtesy of Dr. Wallis JP, Newcastle, UK

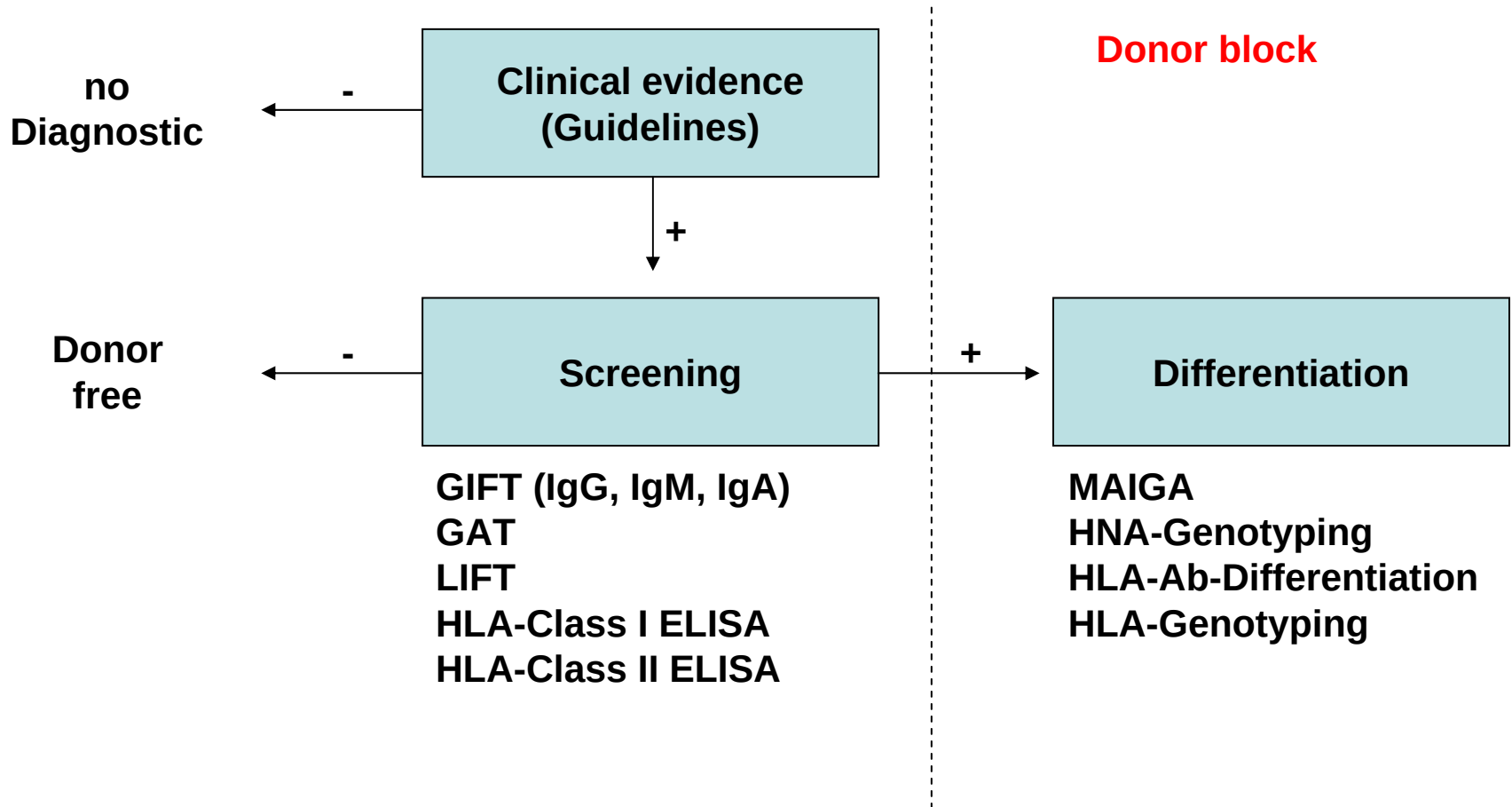
Cell priming and activation in TRALI



TRALI



TRALI: Serological Investigations

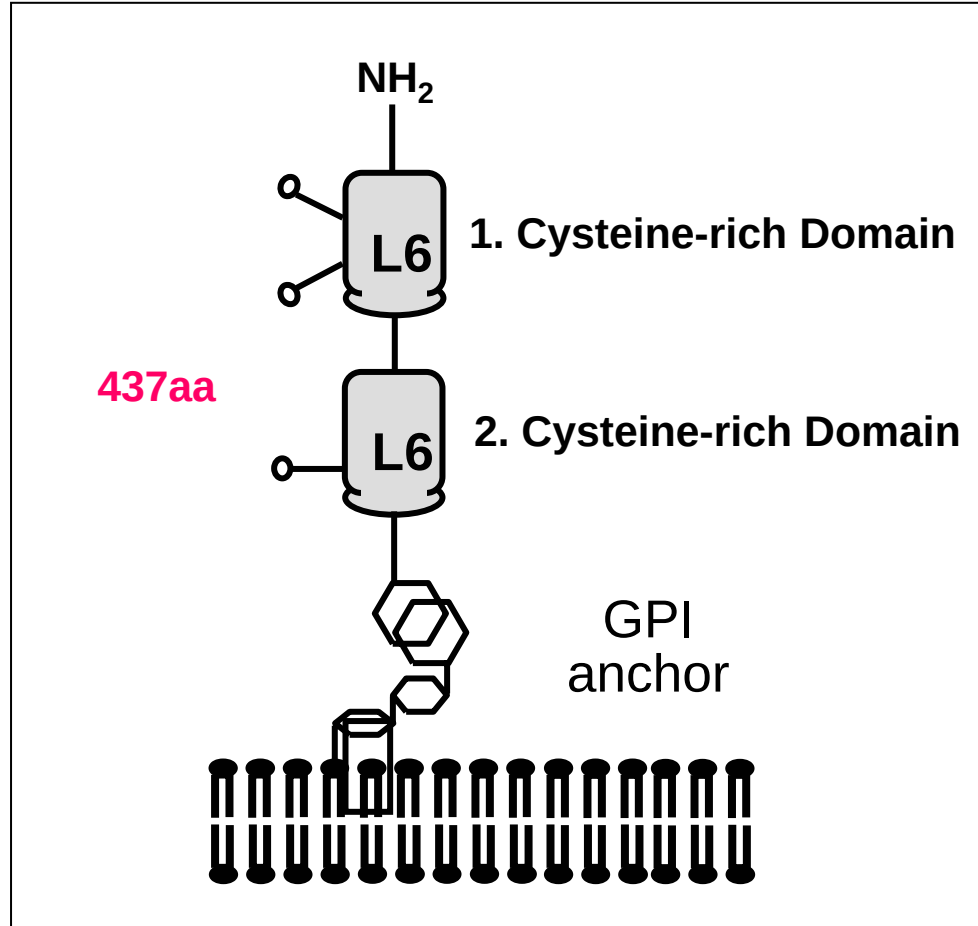


NB1: Current Knowledges

Structure	GPI-anchored Glycoprotein 58-64 kDa Polymorph (NB1 and PRV-1 Allele) Ly-6 Family (uPAR/CD59)
Expression	Neutrophils, not other blood cells Plasma membrane, secondary granules and serum
Immunogenecity	HNA-2a Neutropenia, TRALI
Biomarker	Overexpression in myeloproliferative disorders, bacterial infection, and after G-CSF administration
Function	Mediates surface expression of the ANCA antigen PR3 (neutrophil activation) Bind PECAM-1 (neutrophil transendothelial migration)

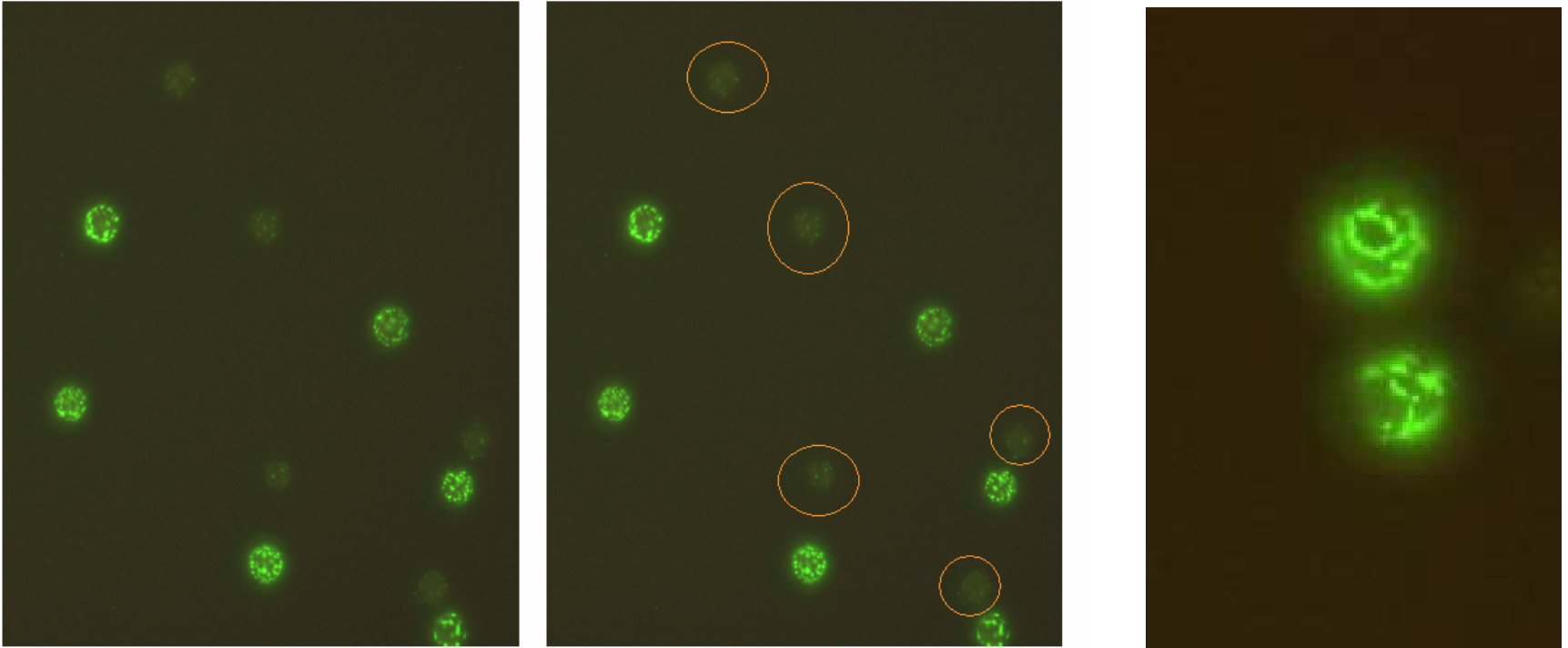
Molecular Structure of NB1 (HNA-2a)

A Member of Ly6 Superfamily



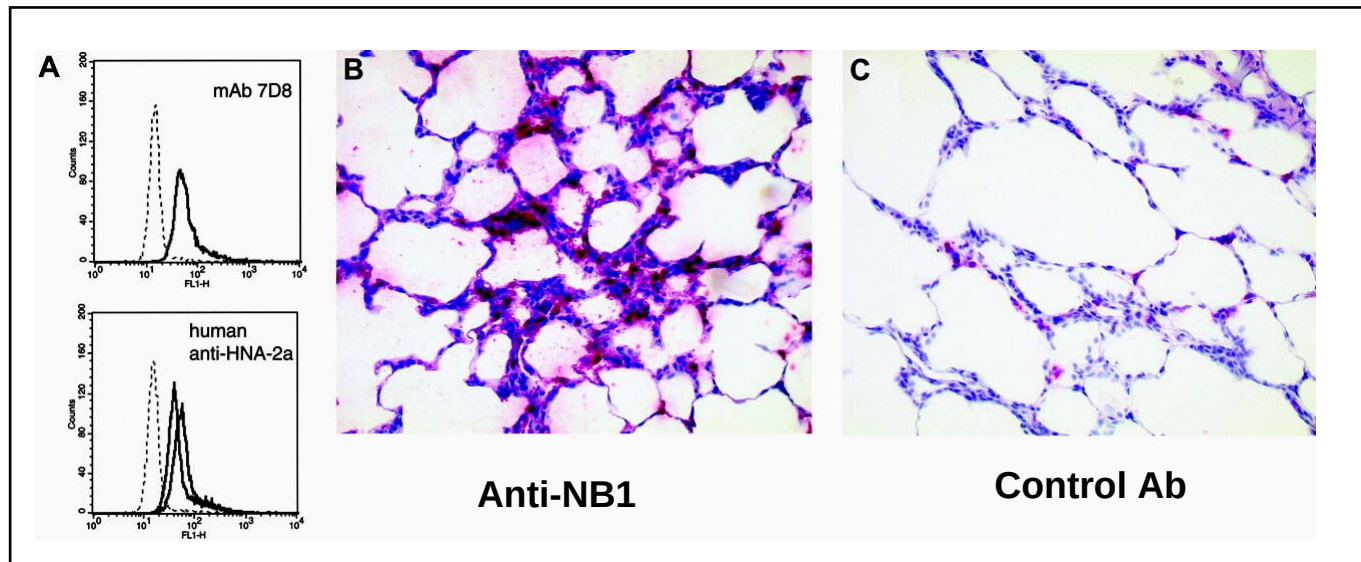
Kissel et al, Eur J Immunol 2001

Cellular pattern of anti-NB1



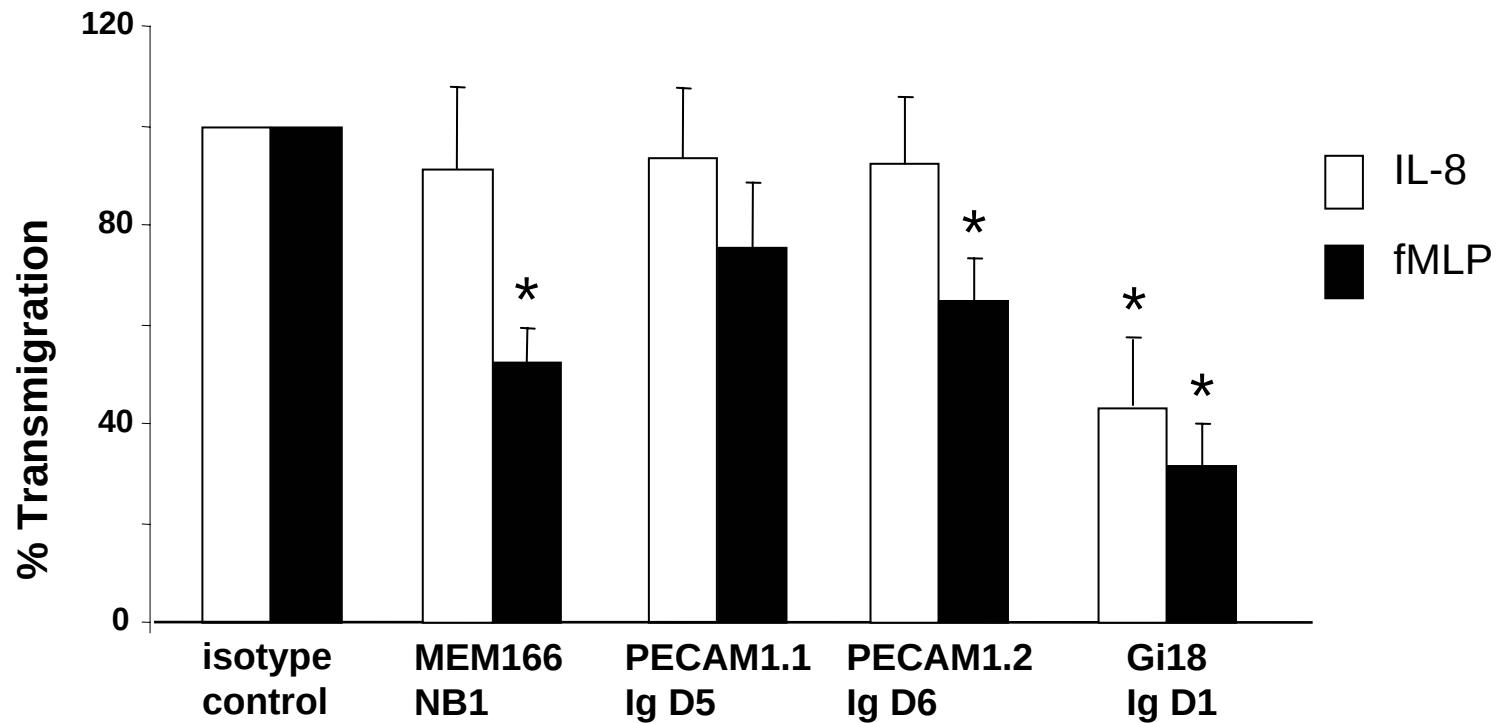
Courtesy of Dr. A. Weil, Hagen

TRALI Induced by anti-NB1: *Ex Vivo* Rat Lung Model



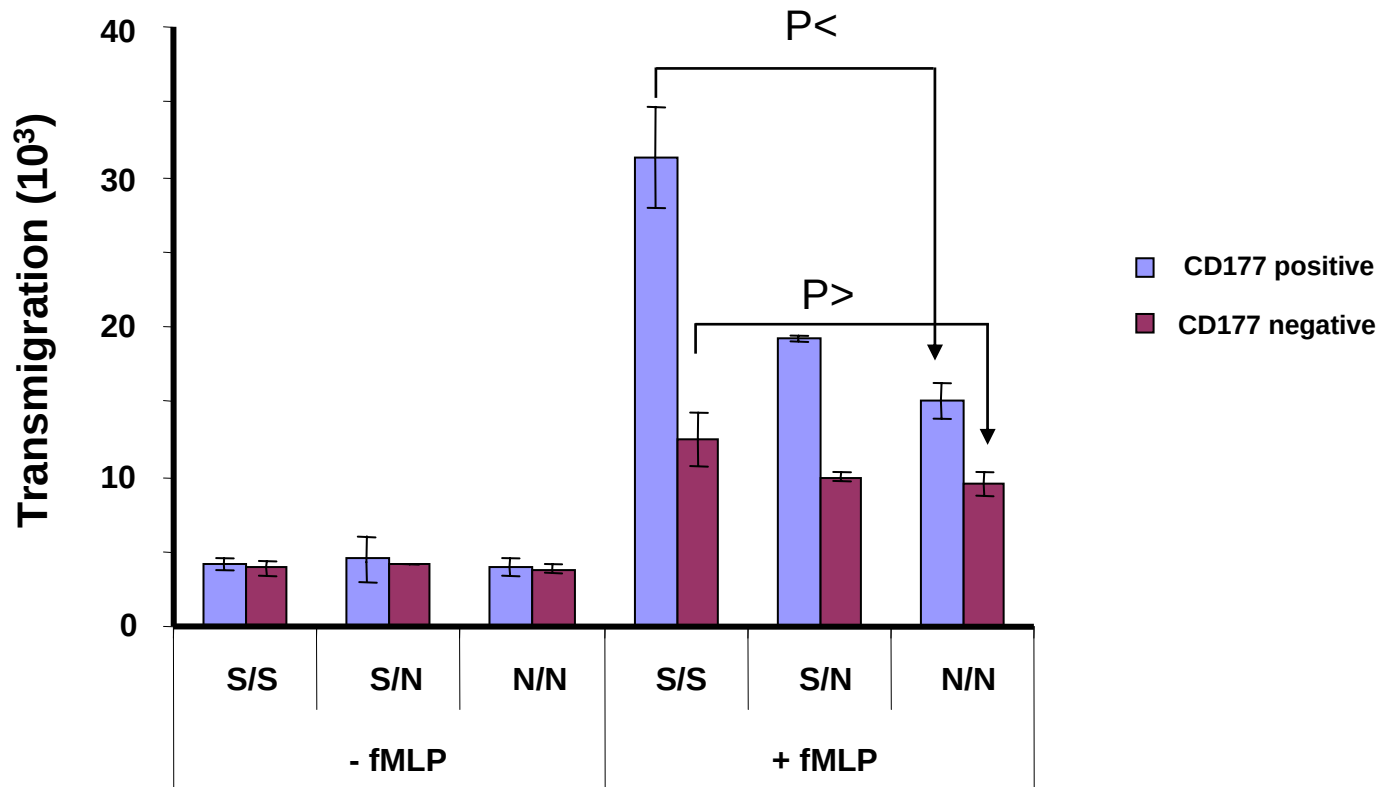
**Anti-NB1 leads to neutrophil activation
and clustering of leukocytes in the capillaries of the lung**

Anti NB1 blocks Neutrophil Migration through Endothelial Cells

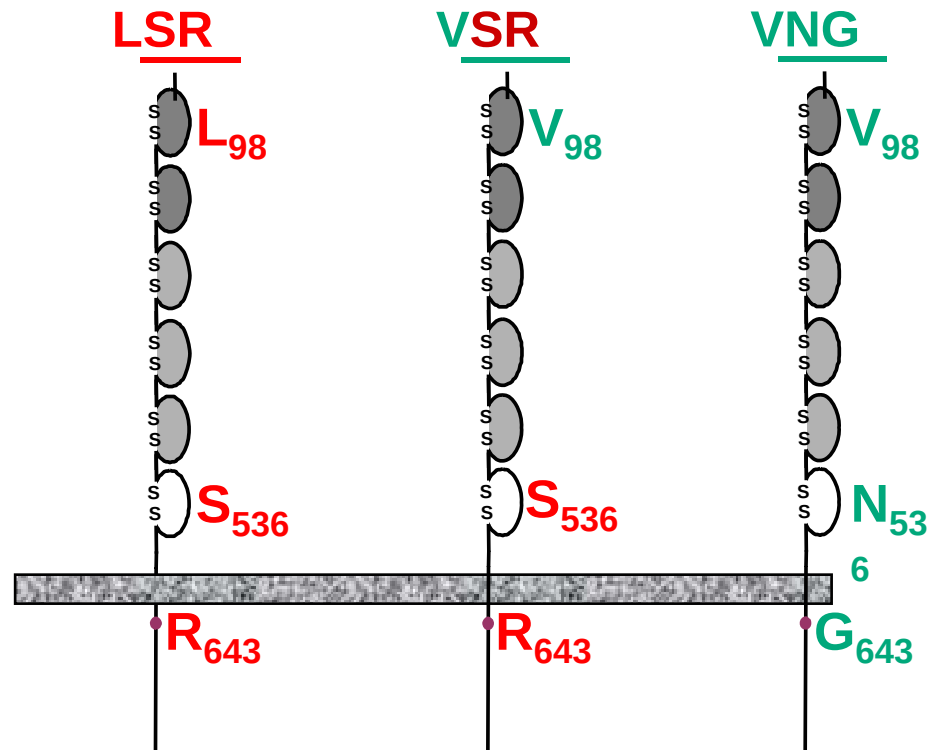


Sachs et al, J Biol Chem 2007

Neutrophil Migration Through PECAM-1 Phenotyped HUVECs



Major PECAM-1 Isoforms

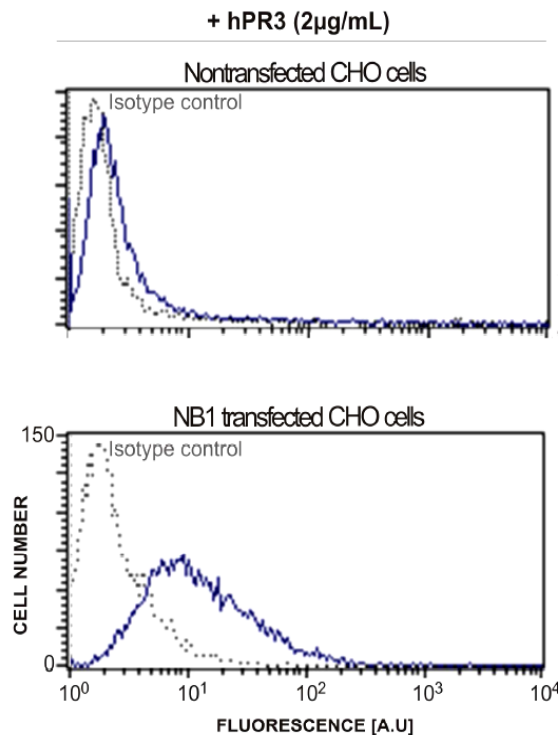


Gene Frequency

Asians (n=15)	.40	.06	.47
Hispanics (n=10)	.65	0	.35
Caucasians (n=62)	.57	.06	.37
Native Americans (n=3)	.67	0	.33
African Americans (n=49)	.38	.42	.19

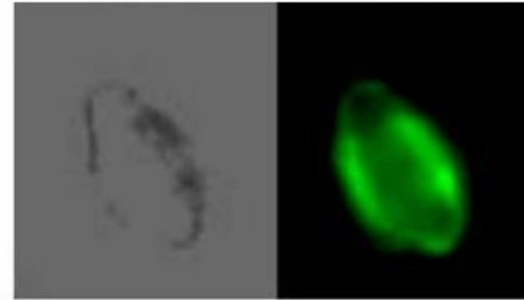
PR3 and CD177 Complex Formation

PR3 surface expression detection

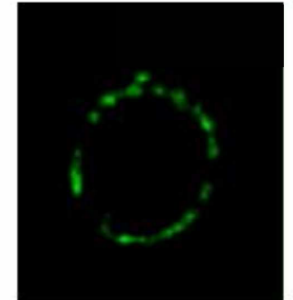


NB1 transfected CHO cells + hPR3 (2 μ g/mL)

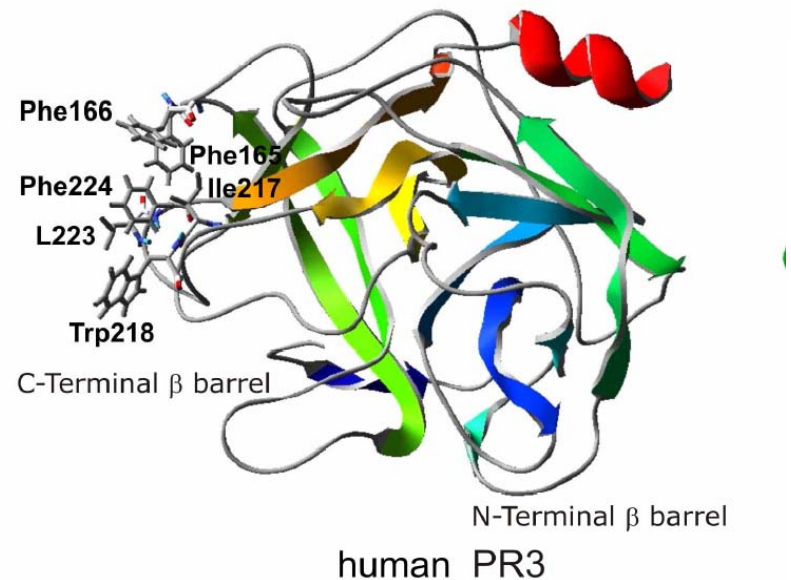
DIC



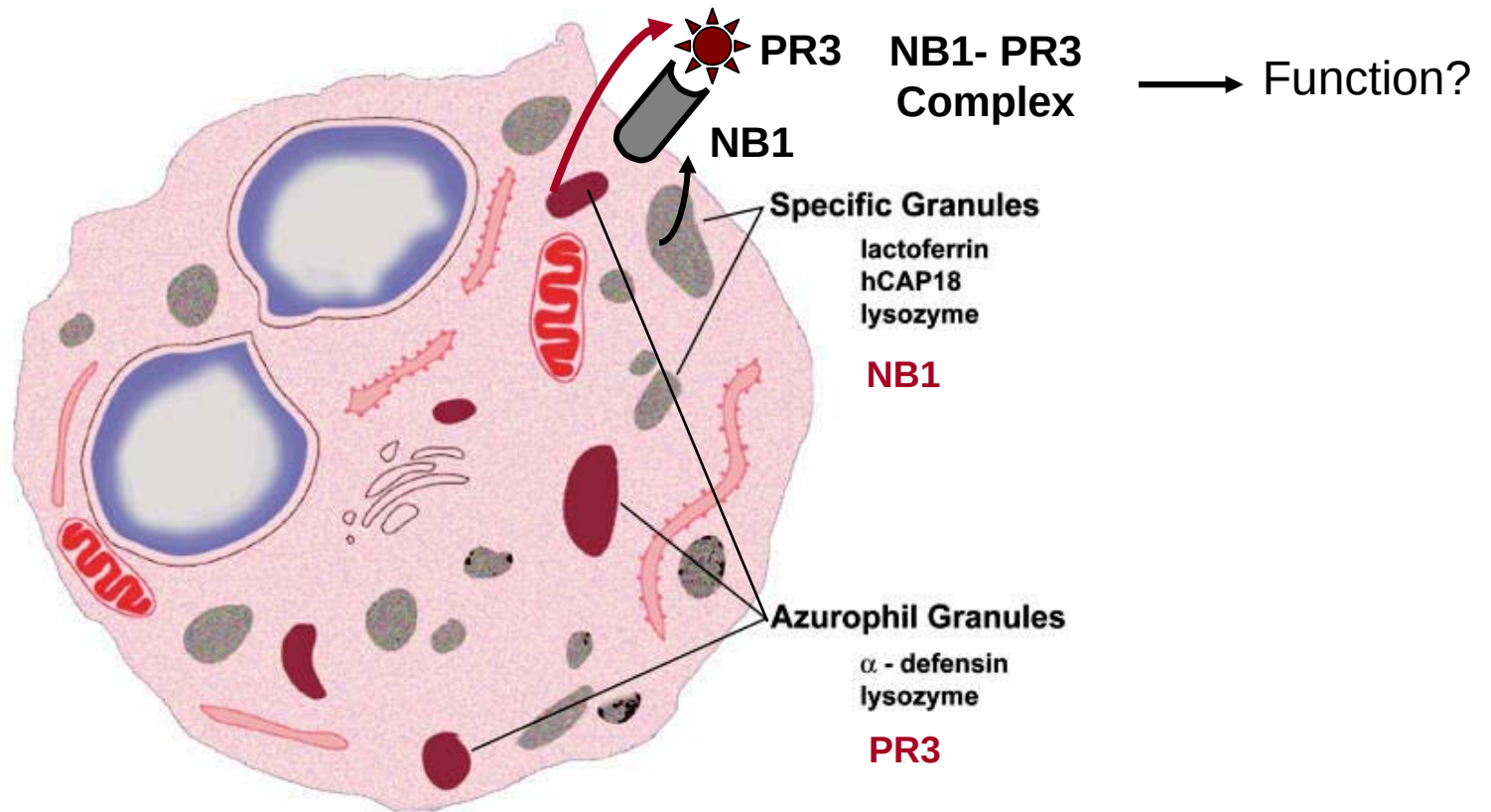
Fluorescent microscopy



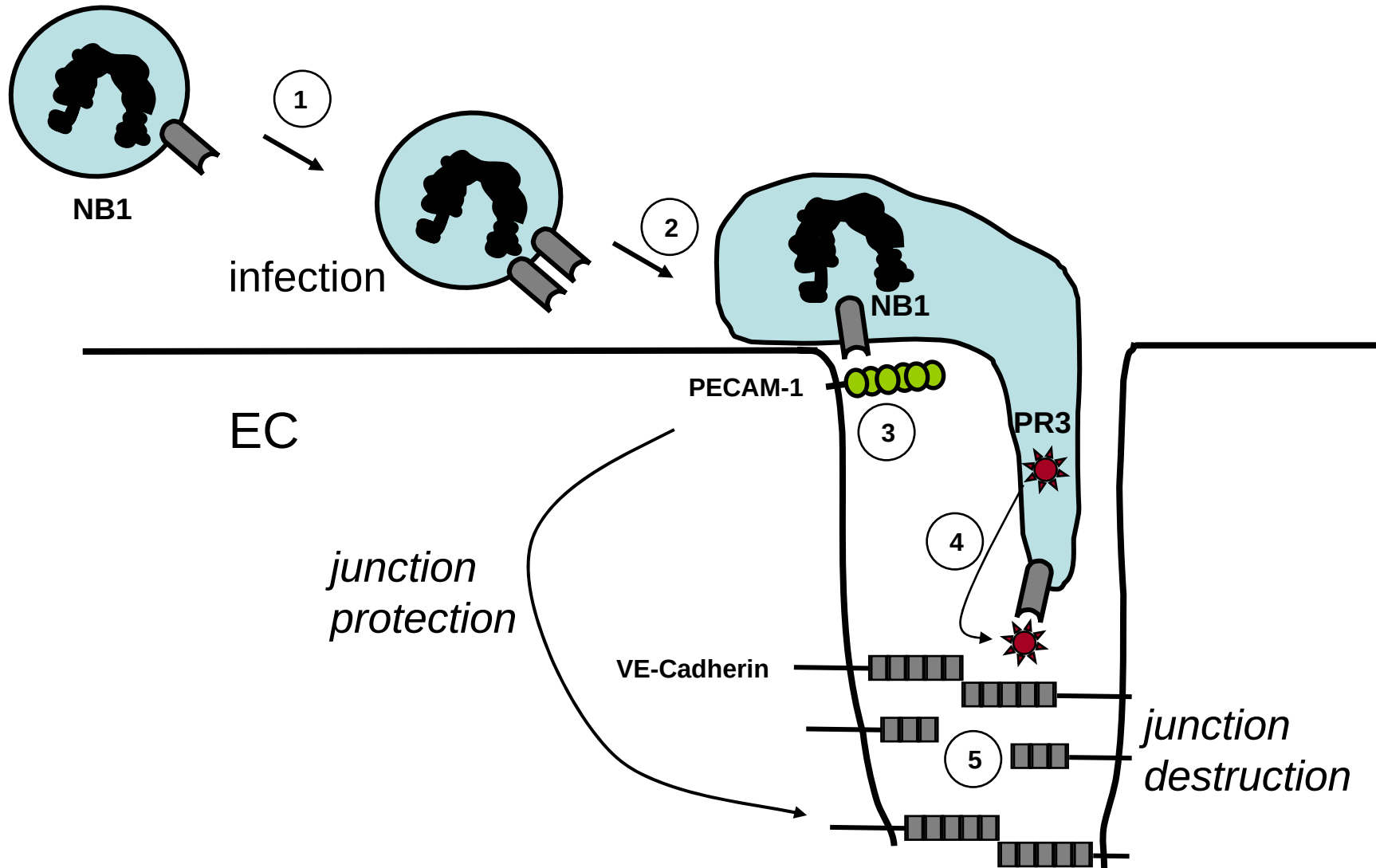
Confocal microscopy



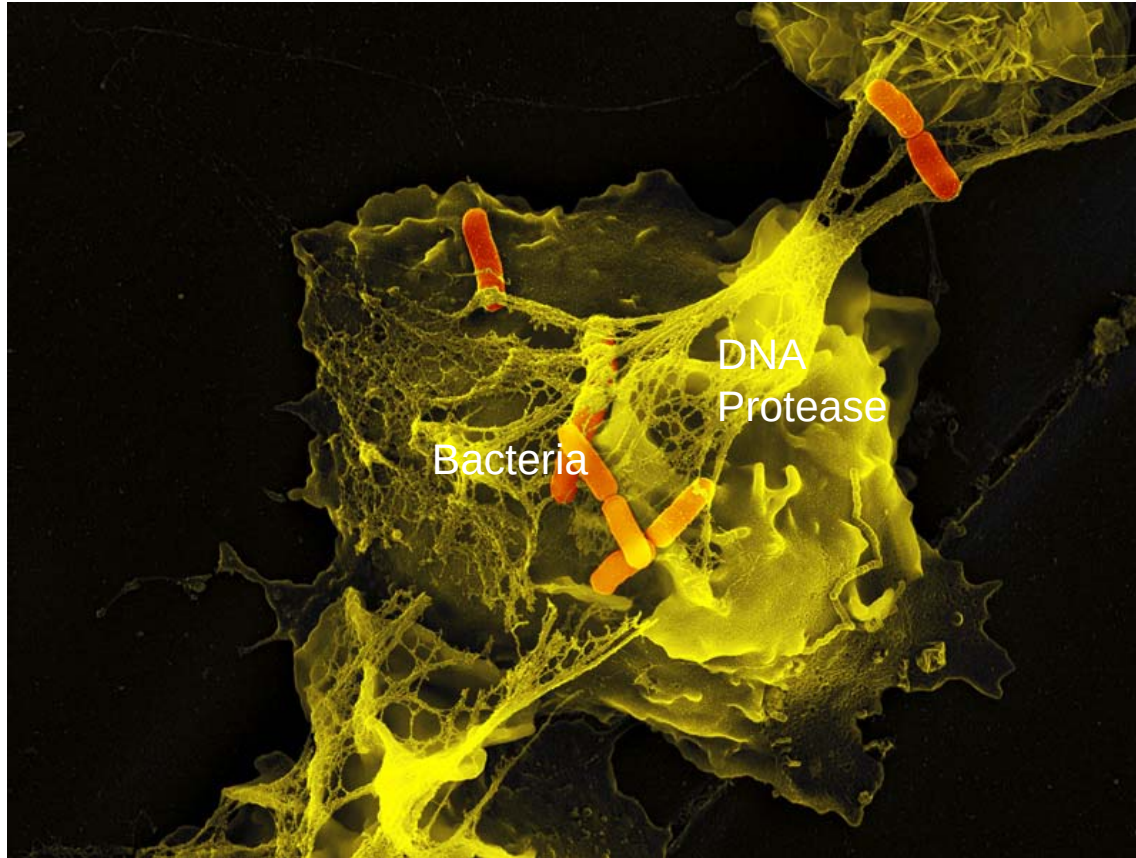
Neutrophil Serin Proteases



The Role of NB1 in Multistep Process of Neutrophil Transendothelial Migration



Neutrophil Extracellular TRAP



Brinkmann et al, Science 2004