



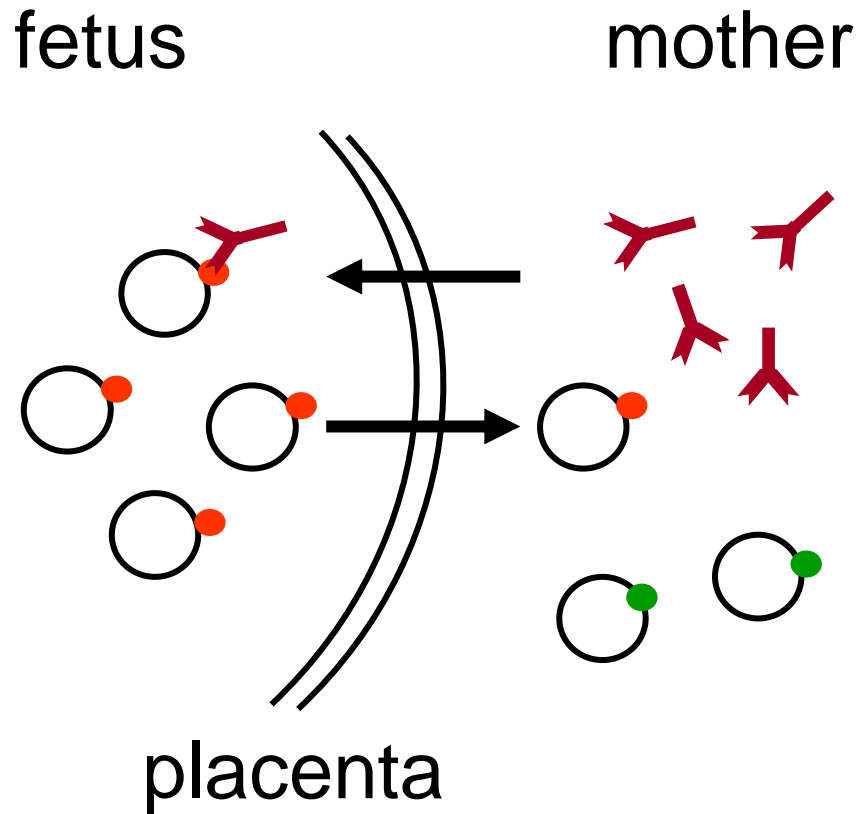
Neutrophil receptors/antigens and their role in immune-mediated neutropenias

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*Training School on Autoimmune Neutropenias 2020/2021
Organized by Carlo Dufour & Francesca Fioredda, Genova, Italy*

Pathomechanism of NIN



● paternal antigen

○ neutrophil

Neonatal immune neutropenia (NIN) is the neutrophil equivalent of hemolytic disease of the fetus and newborn (HDFN).

Main differences: neutropenia has **no** clinical impact on the fetus; plus, clinical manifestation of neutropenia after birth is **rare**.

There are **no markers** to predict whether the newborn will suffer from an infection. There is no impact of: neutrophil nadir (= lowest count), age of gestation, birth weight, **specificity of the antibody**.

Infections are usually **mild** (skin), very rarely severe (sepsis).

Human neutrophil antigens (HNA)

GP
FcγRIIIb (CD16b)
CD177
CTL2
Mac-1 (CD11b)
LFA-1 (CD11a)

Typical antibody specificities in Europe

Specificity	Amsterdam, The Netherlands (n=35)	Giessen, Germany (n=67)
HNA-1a	20%	45%
HNA-1b	34%	30%
HNA-1c	6%	6%
HNA-1d	0	0
CD16b	11%	7%
HNA-2	23%	10%
HNA-3a	3%	2%
HNA-4a	0	0
HNA-5a	3%	0

Genetics of human neutrophil antigens (HNA)

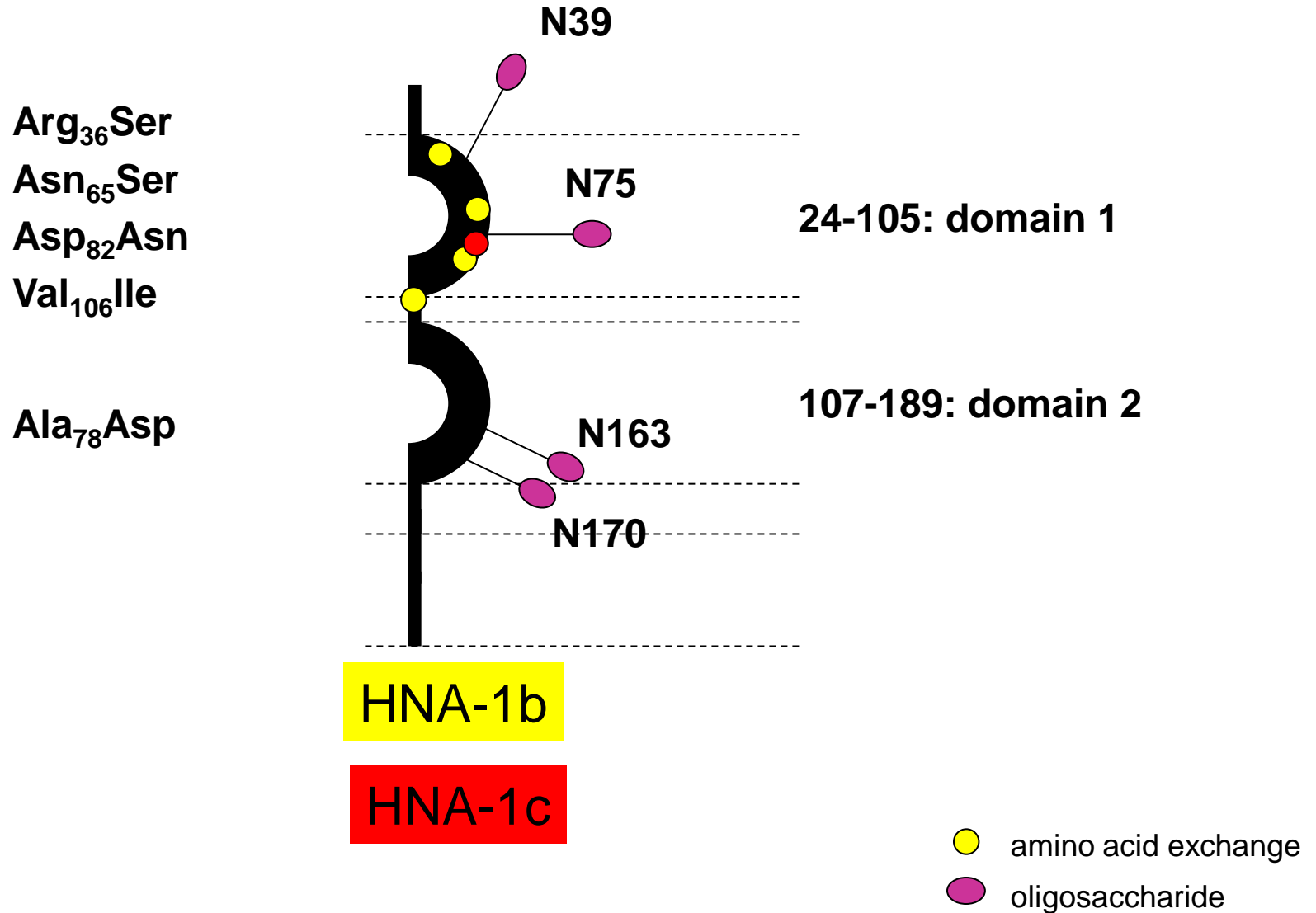
HNA	GP	Antigen		Allele	Allele defining base exchanges					
1	FcγRIIb (CD16b)	HNA-1a	NA1	FCGR3B*01	108G	114C	194A	233C	244G	316G
		HNA-1b	NA2	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1c	SH	FCGR3B*03	108C	114T	194G	233A	244A	316A
		HNA-1d	-	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1null	-	FCRG3B*null	<i>gene depletion</i>					
2	CD177	HNA-2	NB1	CD177*01	843A	1011G				
		<i>HNA-2null</i>	-	<i>CD177*02</i>	843T	1011G				
		<i>HNA-2null</i>	-	<i>CD177*03</i>	843A	1011Δ G				
3	CTL2	HNA-3a	5b	SLC44A2*01	451C	455G				
		HNA-3a	5b	SLC44A2*03	451T	455G				
		HNA-3b*	-	SLC44A2*02	451C	455A				
4	Mac-1 (CD11b)	HNA-4a	Mart	ITGAM*01	230G					
		HNA-4b	-	ITGAM*02	230A					
5	LFA-1 (CD11a)	HNA-5a	Ond	ITGAL*01	2372G					
		-	-	ITGAL*02	2372C					

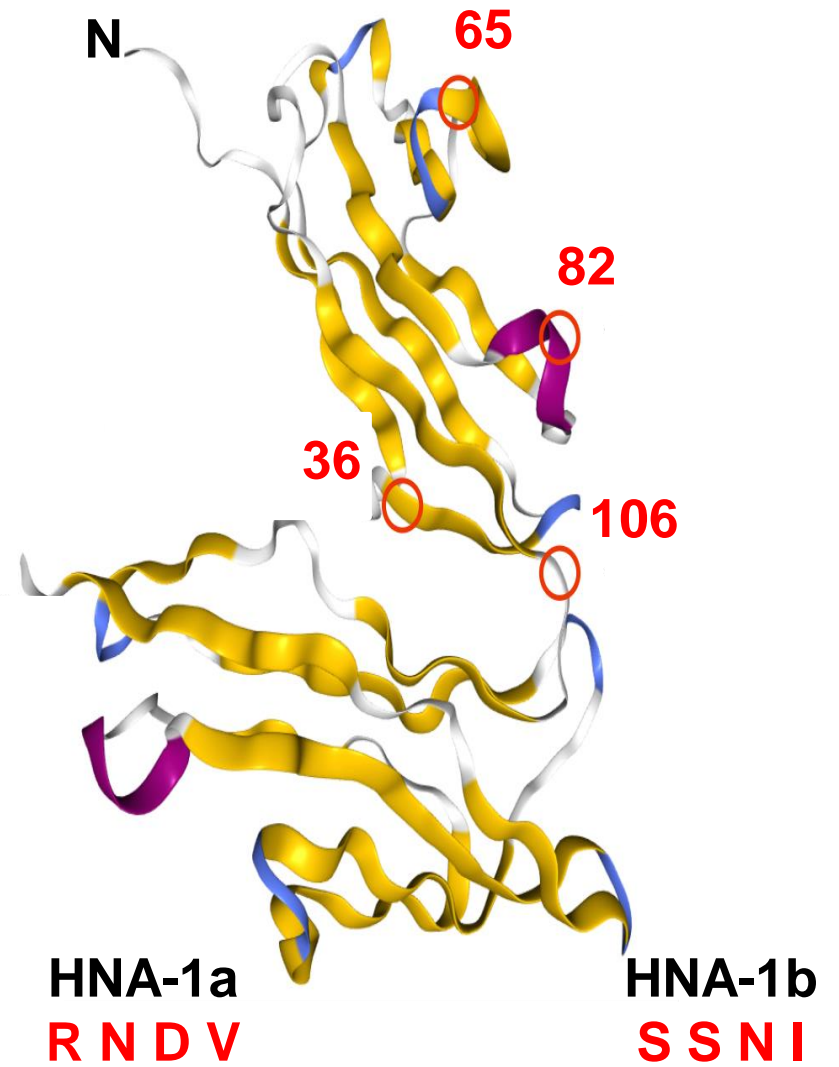
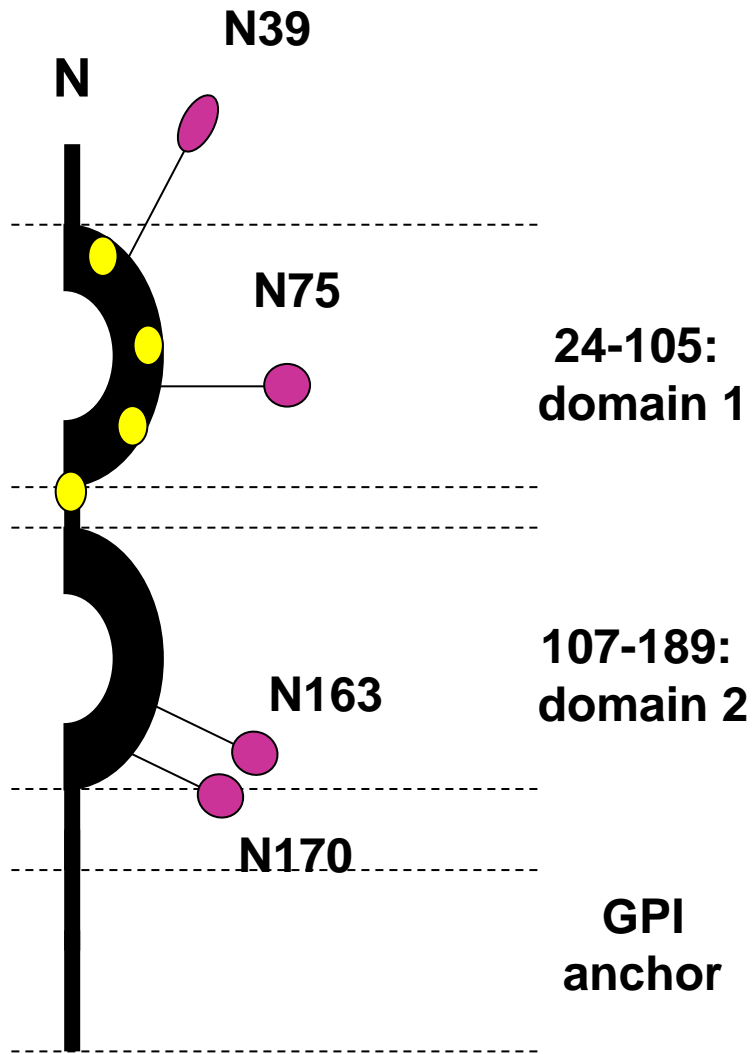
Human neutrophil antigens (HNA)

HNA	GP	Antigen		Allel	Allel-definierende Basenaustausche					
1	FcγRIIIb (CD16b)	HNA-1a	NA1	FCGR3B*01	108G	114C	194A	233C	244G	316G
		HNA-1b	NA2	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1c	SH	FCGR3B*03	108C	114T	194G	233A	244A	316A
		HNA-1d	-	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1null	-	FCRG3B*null	<i>gene depletion</i>					
2	CD177	HNA-2	NB1	CD177*01	843A	1011G				
		HNA-2null	-	CD177*02	843T	1011G				
		HNA-2null	-	CD177*03	843A	1011Δ G				
3	CTL2	HNA-3a	5b	SLC44A2*01	451C	455G				
		HNA-3a	5b	SLC44A2*03	451T	455G				
		HNA-3b*	-	SLC44A2*02	451C	455A				
4	Mac-1 (CD11b)	HNA-4a	Mart	ITGAM*01	230G					
		HNA-4b	-	ITGAM*02	230A					
5	LFA-1 (CD11a)	HNA-5a	Ond	ITGAL*01	2372G					
		-	-	ITGAL*02	2372C					

HNA-1 resides on FcγRIIIb (CD16b)

This receptor is only present on neutrophils!



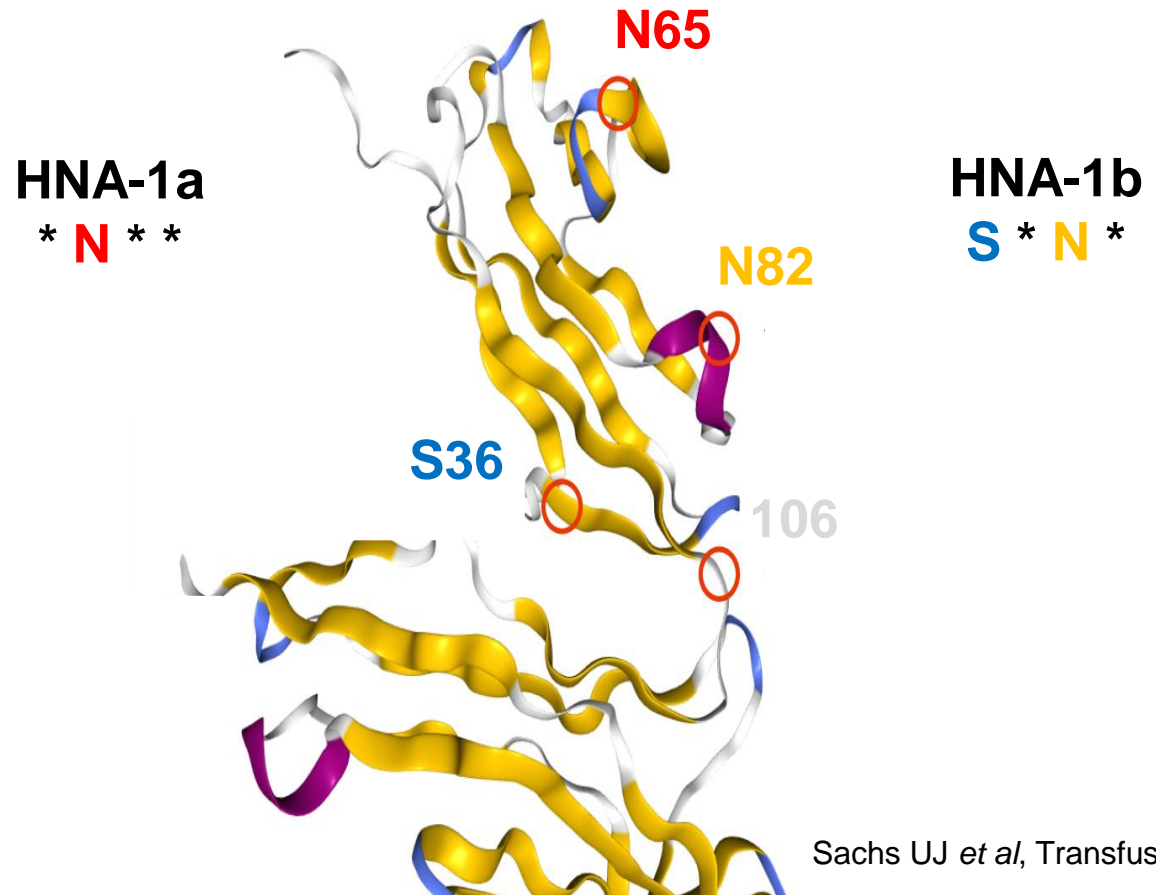


Epitope composition is not antithetical.

N65 alone determines the presence of HNA-1a.

S36 and/or **N82** determine the presence of HNA-1b.

AA 106 is irrelevant.



Human neutrophil antigens (HNA)

HNA	GP	Antigen		Allel	Allel-definierende Basenaustausche					
1	FcγRIIb (CD16b)	HNA-1a	NA1	FCGR3B*01	108G	114C	194A	233C	244G	316G
		HNA-1b	NA2	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1c	SH	FCGR3B*03	108C	114T	194G	233A	244A	316A
		HNA-1d	-	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1null	-	FCRG3B*null	<i>gene depletion</i>					
2	CD177	HNA-2	NB1	CD177*01	843A	1011G				
		<i>HNA-2null</i>	-	<i>CD177*02</i>	843T	1011G				
		<i>HNA-2null</i>	-	<i>CD177*03</i>	843A	1011Δ G				
3	CTL2	HNA-3a	5b	SLC44A2*01	451C	455G				
		HNA-3a	5b	SLC44A2*03	451T	455G				
		HNA-3b*	-	SLC44A2*02	451C	455A				
4	Mac-1 (CD11b)	HNA-4a	Mart	ITGAM*01	230G					
		HNA-4b	-	ITGAM*02	230A					
5	LFA-1 (CD11a)	HNA-5a	Ond	ITGAL*01	2372G					
		-	-	ITGAL*02	2372C					

Human neutrophil antigens (HNA)

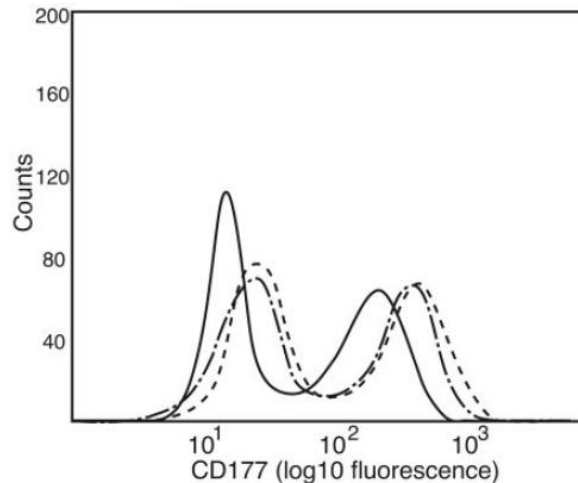
HNA	GP	Antigen		Allel	Allel-definierende Basenaustausche					
1	FcγRIIb (CD16b)	HNA-1a	NA1	FCGR3B*01	108G	114C	194A	233C	244G	316G
		HNA-1b	NA2	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1c	SH	FCGR3B*03	108C	114T	194G	233A	244A	316A
		HNA-1d	-	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1null	-	FCRG3B*null	<i>gene depletion</i>					
2	CD177	HNA-2	NB1	CD177*01	843A	1011G				
		HNA-2null	-	CD177*02	843T	1011G				
		HNA-2null	-	CD177*03	843A	1011Δ G				
3	CTL2	HNA-3a	5b	SLC44A2*01	451C	455G				
		HNA-3a	5b	SLC44A2*03	451T	455G				
		HNA-3b*	-	SLC44A2*02	451C	455A				
4	Mac-1 (CD11b)	HNA-4a	Mart	ITGAM*01	230G					
		HNA-4b	-	ITGAM*02	230A					
5	LFA-1 (CD11a)	HNA-5a	Ond	ITGAL*01	2372G					
		-	-	ITGAL*02	2372C					

HNA-2 = CD177

CD177, NB1, HNA-2, PRV-1, **only** on neutrophils and their progenitors (secondary granules & surface marker).

GPI-anchored GP, member of Ly-6 gene family, function largely unknown. Carries proteinase 3. Possibly involved in cell maturation and/or migration.

Heterogeneous expression, often **bimodal**. Some individuals (< 3%) do not carry HNA-2 at all (**HNA-2 null**).



Immunisation of HNA-2-null against HNA-2 leads to the formation of **iso-antibodies**, anti-HNA-2 (*not* “HNA-2a”).

Human neutrophil antigens (HNA)

HNA	GP	Antigen		Allel	Allel-definierende Basenaustausche					
1	FcγRIIb (CD16b)	HNA-1a	NA1	FCGR3B*01	108G	114C	194A	233C	244G	316G
		HNA-1b	NA2	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1c	SH	FCGR3B*03	108C	114T	194G	233A	244A	316A
		HNA-1d	-	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1null	-	FCRG3B*null	<i>gene depletion</i>					
2	CD177	HNA-2	NB1	CD177*01	843A	1011G				
		<i>HNA-2null</i>	-	<i>CD177*02</i>	843T	1011G				
		<i>HNA-2null</i>	-	<i>CD177*03</i>	843A	1011Δ G				
3	CTL2	HNA-3a	5b	SLC44A2*01	451C	455G				
		HNA-3a	5b	SLC44A2*03	451T	455G				
		HNA-3b*	-	SLC44A2*02	451C	455A				
4	Mac-1 (CD11b)	HNA-4a	Mart	ITGAM*01	230G					
		HNA-4b	-	ITGAM*02	230A					
5	LFA-1 (CD11a)	HNA-5a	Ond	ITGAL*01	2372G					
		-	-	ITGAL*02	2372C					

Human neutrophil antigens (HNA)

HNA	GP	Antigen		Allel	Allel-definierende Basenaustausche					
1	FcγRIIb (CD16b)	HNA-1a	NA1	FCGR3B*01	108G	114C	194A	233C	244G	316G
		HNA-1b	NA2	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1c	SH	FCGR3B*03	108C	114T	194G	233A	244A	316A
		HNA-1d	-	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1null	-	FCRG3B*null	<i>gene depletion</i>					
2	CD177	HNA-2	NB1	CD177*01	843A	1011G				
		<i>HNA-2null</i>	-	<i>CD177*02</i>	843T	1011G				
		<i>HNA-2null</i>	-	<i>CD177*03</i>	843A	1011Δ G				
3	CTL2	HNA-3a	5b	SLC44A2*01	451C	455G				
		HNA-3a	5b	SLC44A2*03	451T	455G				
		HNA-3b*	-	SLC44A2*02	451C	455A				
4	Mac-1 (CD11b)	HNA-4a	Mart	ITGAM*01	230G					
		HNA-4b	-	ITGAM*02	230A					
5	LFA-1 (CD11a)	HNA-5a	Ond	ITGAL*01	2372G					
		-	-	ITGAL*02	2372C					

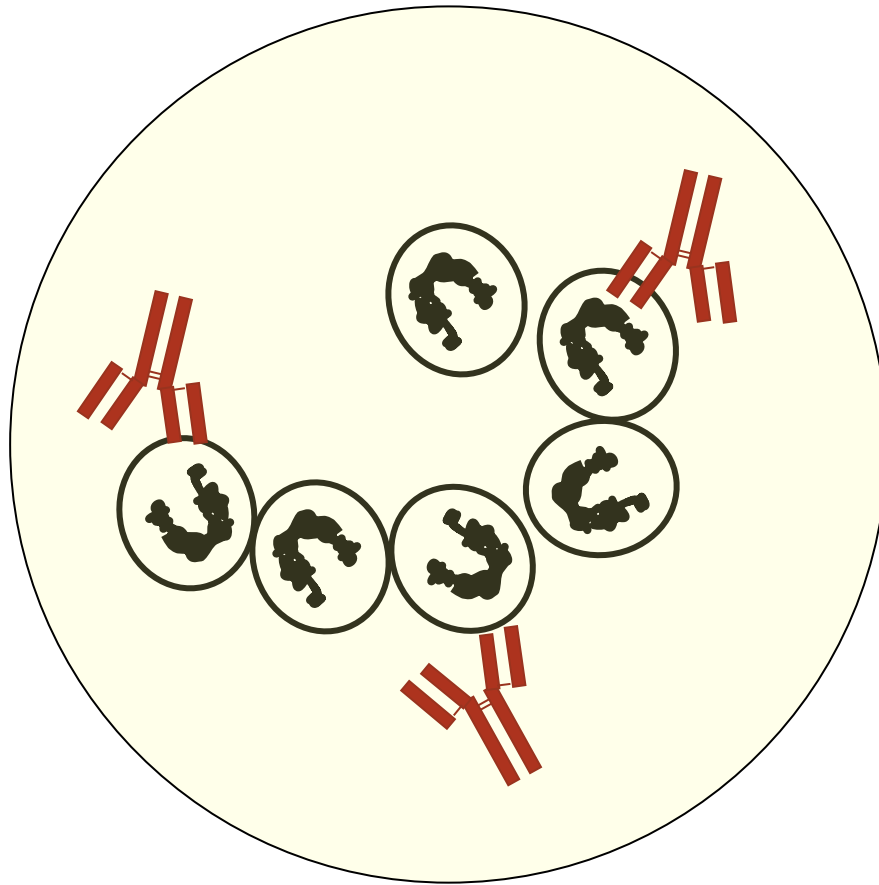


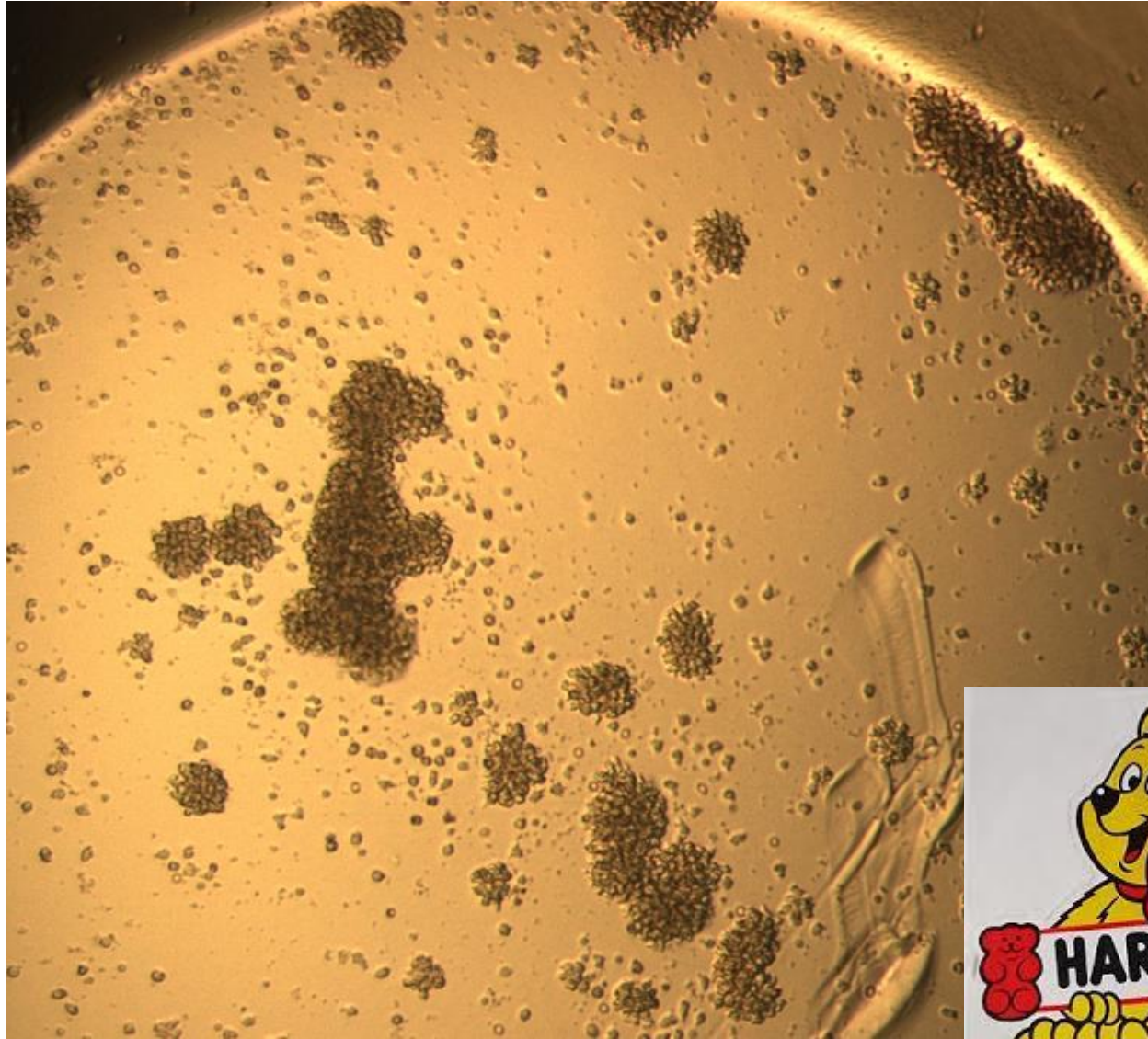
GAT = 0



GAT = 80

Granulozytenagglutinationstest (GAT)





Human neutrophil antigens (HNA)

HNA	GP	Antigen		Allel	Allel-definierende Basenaustausche					
1	FcγRIIb (CD16b)	HNA-1a	NA1	FCGR3B*01	108G	114C	194A	233C	244G	316G
		HNA-1b	NA2	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1c	SH	FCGR3B*03	108C	114T	194G	233A	244A	316A
		HNA-1d	-	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1null	-	FCRG3B*null	<i>gene depletion</i>					
2	CD177	HNA-2	NB1	CD177*01	843A	1011G				
		<i>HNA-2null</i>	-	<i>CD177*02</i>	843T	1011G				
		<i>HNA-2null</i>	-	<i>CD177*03</i>	843A	1011Δ G				
3	CTL2	HNA-3a	5b	SLC44A2*01	451C	455G				
		HNA-3a	5b	SLC44A2*03	451T	455G				
		HNA-3b*	-	SLC44A2*02	451C	455A				
4	Mac-1 (CD11b)	HNA-4a	Mart	ITGAM*01	230G					
		HNA-4b	-	ITGAM*02	230A					
5	LFA-1 (CD11a)	HNA-5a	Ond	ITGAL*01	2372G					
		-	-	ITGAL*02	2372C					

Human neutrophil antigens (HNA)

HNA	GP	Antigen		Allel	Allel-definierende Basenaustausche					
1	FcγRIIb (CD16b)	HNA-1a	NA1	FCGR3B*01	108G	114C	194A	233C	244G	316G
		HNA-1b	NA2	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1c	SH	FCGR3B*03	108C	114T	194G	233A	244A	316A
		HNA-1d	-	FCGR3B*02	108C	114T	194G	233C	244A	316A
		HNA-1null	-	FCRG3B*null	<i>gene depletion</i>					
2	CD177	HNA-2	NB1	CD177*01	843A	1011G				
		<i>HNA-2null</i>	-	<i>CD177*02</i>	843T	1011G				
		<i>HNA-2null</i>	-	<i>CD177*03</i>	843A	1011Δ G				
3	CTL2	HNA-3a	5b	SLC44A2*01	451C	455G				
		HNA-3a	5b	SLC44A2*03	451T	455G				
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4	Mac-1 (CD11b)	HNA-4a	Mart	ITGAM*01	230G					
		HNA-4b	-	ITGAM*02	230A					
5	LFA-1 (CD11a)	HNA-5a	Ond	ITGAL*01	2372G					
		-	-	ITGAL*02	2372C					

Let's talk about these antibodies in

AUTOIMMUNE NEUTROPENIA

How do we know that autoantibodies are causative?

disappearance of **sensitized neutrophils** after the injection of rabbit anti-guinea pig antibodies

[Lawrence *et al*, J Lab Clin Med 1967; Simpson & Ross, Am J Pathol 1971]

in AIN, antibodies are **frequently detected** (80%-100%), both attached to neutrophils and as free antibodies in serum

[Lalezari *et al*, J Pediatr 1986; Bux *et al* Blood 1998]

circulating immune complexes (CICs) are sometimes observed (11/25) but are not related to GAT; or they are not detected at all (8/8)

[Lalezari *et al*, J Pediatr 1986; Kobayashi *et al*, J Pediatr Hem/Onc 2003]

autoimmunity to neutrophils appears to be of **restricted clonal origin** (5/6 in chronic idiopathic neutropenia)

[Shastri *et al*, Am J Hematol 1991]

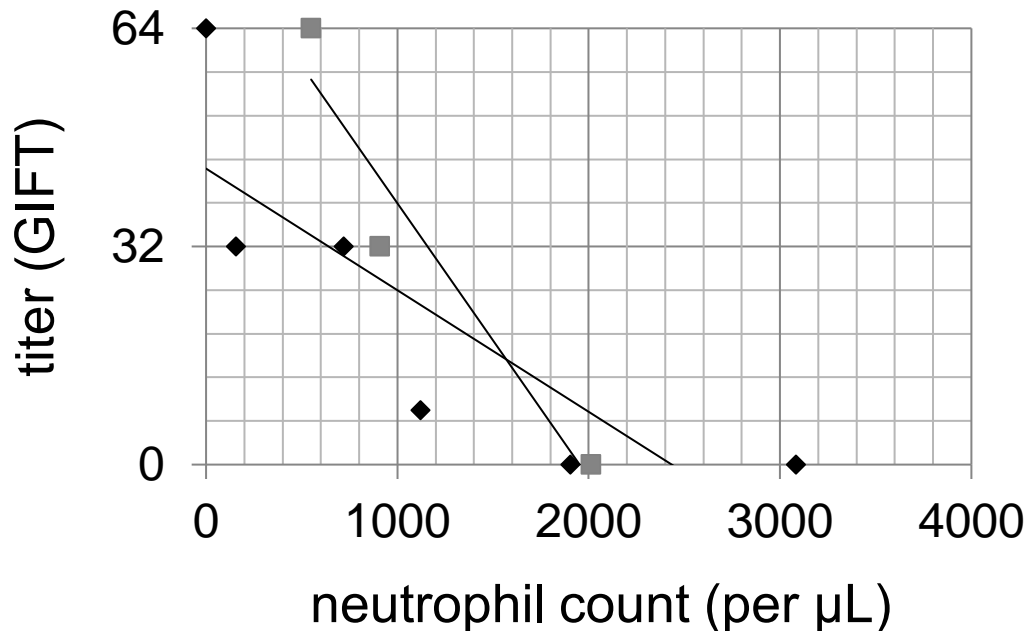
How do we know that autoantibodies are causative?

Duration of AIN (until spontaneous resolution) is **dependent on antibody strength** at the time of diagnosis

[Kobayashi *et al*, Blood 2002]

Antibodies may gradually decline in titer, and both direct GIFT (16/18 and 4/4) and indirect GIFT (17/17; 30/30) become **negative with recovery** of AIN.

[Conway *et al*, Pediatrics 1987 ; Yamada *et al*, Pediatr Int 2002; Taniuchi *et al*, Acta Paediatr 2002; Bux *et al*, Blood 1998]



Is AIN associated with Fc-receptor polymorphisms?

Receptor	Genotype	AIN			healthy controls	
		Audrain, 2011 (France)	Taniuchi, 2001 (Japan)	Wang, 2009 (Taiwan)	Audrain, 2011 (France)	Taniuchi, 2001 (Japan)
FcγRIIIb	HNA-1aa	41	83	84	13	30
	HNA-1bb	14	0	3	40	10
	HNA-1ab	44	18	13	47	60
	<i>a allele fq.</i>	0.64	0.91	0.91	0.37	0.60
	<i>b allele fq.</i>	0.36	0.09	0.09	0.64	0.40
FcγRIIa	131H/H	34	100	--	30	63
	131R/R	14	0	--	20	2
	131H/R	52	0	--	50	35
	<i>H allele fq.</i>	0.60	1.00	--	0.55	0.81
	<i>R allele fq.</i>	0.40	0.00	--	0.45	0.19

Is AIN associated with Fc-receptor polymorphisms?

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	<i>a allele fq.</i>	0.64	0.91	0.91	0.37	0.60
	<i>b allele fq.</i>	0.36	0.09	0.09	0.64	0.40
FcγRIIa	131H/H	34	100	--	30	63
	131R/R	14	0	--	20	2
	131H/R	52	0	--	50	35
	<i>H allele fq.</i>	0.60	1.00	--	0.55	0.81
	<i>R allele fq.</i>	0.40	0.00	--	0.45	0.19

Are there inheritable (genetic) risk factors for AIN?

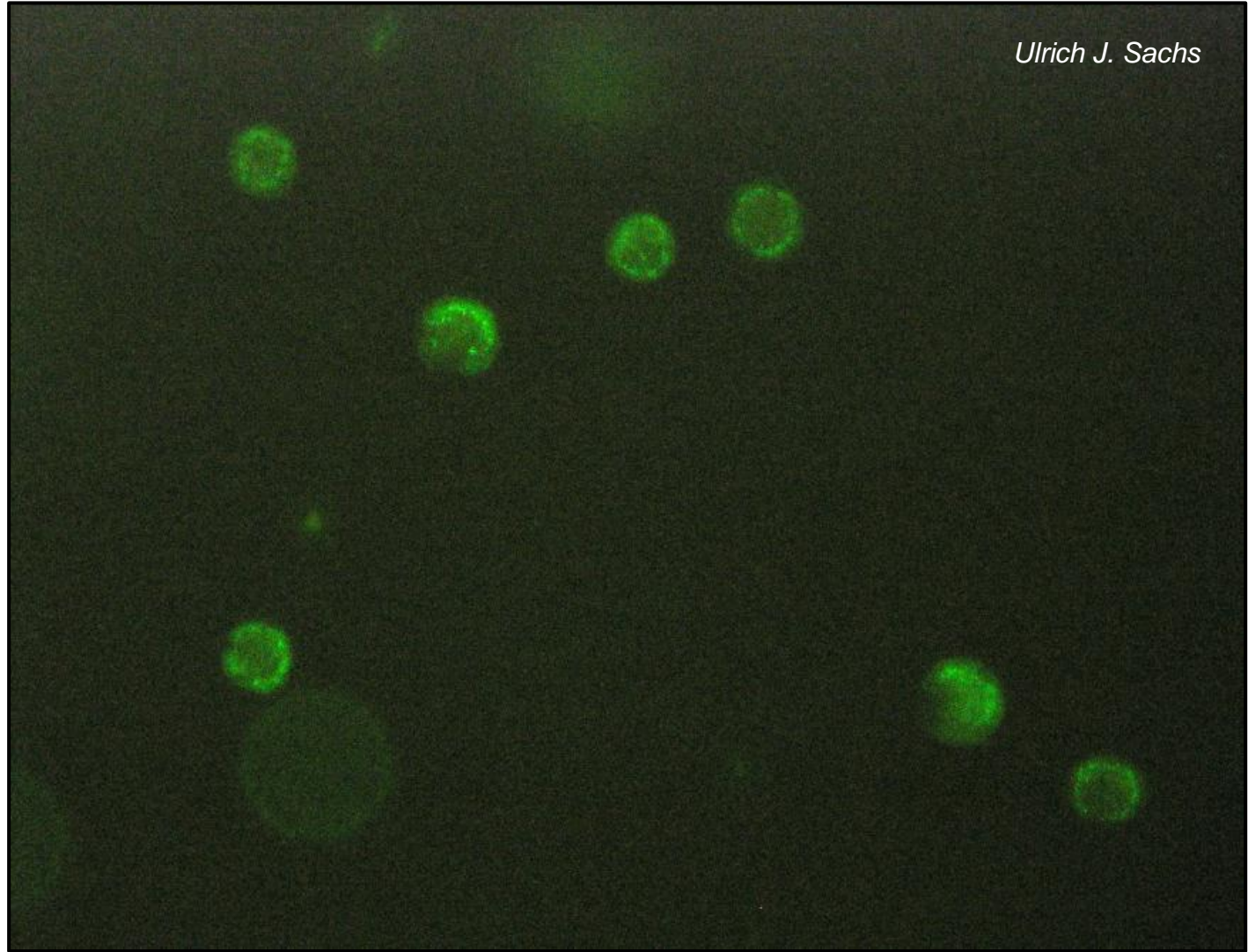
some association with **Fc γ R polymorphisms**, especially with HNA-1a

[Rascu *et al*, Ann N Y Acad Sci 1997; Audrain *et al*, Pediatr Allergy Immunol 2011; Wang *et al*, Transfusion 2009; Taniuchi *et al*, Hum Immunol 2001]

Are autoantibodies of anti-HNA-1a specificity?

study	Audrian, 2011	Bux, 1998		Bruin, 1999
technique	MAIGA	GIFT	MAIGA	GIFT
specificity:				
HNA-1a	40/54	74/240	--	16/21
HNA-1b	5/54	7/240	--	2/21
CD16b	9/54	34/240	34/125	1/21
CD11b/CD18	--	--	26/125	
CR1 (CD35)	--	--	18/125	
Fc γ RII (CD32)	--	--	3/125	
none	--	159/240	--	2/21

GIFT (an indirect immunofluorescence test)



Are autoantibodies of anti-HNA-1a specificity?

Bruin <i>et al</i> , Blood 1999 (n=16)					Giessen laboratory (n=77)				
GIFT	MAIGA (3G8, BW209, MEM154)				GIFT	MAIGA (3G8, BW209)			
	HNA-1a	HNA-1b	both	none		HNA-1a	HNA-1b	both	none
1a (13/16)	3	2	2	6	1a (68/77)	28	19	16	5
1b (2/16)	0	2	0	0	1b (9/77)	3	5	0	1
pan (1/16)	0	0	1	0	pan (0/77)	0	0	0	0

81-83% appear as anti-HNA-1a in GIFT

59-77% have a different “pseudo-“specificity in MAIGA

results in GAT are frequently discordant to GIFT results

[Kobayashi *et al*, Pediatr Res 1989; Giessen AIN study, unpublished]

reactivity in immunoblotting was discordant to GIFT in 6/11 patients

[Taniuchi *et al*, Hum Immunol 2001]

How about the other specificities?

study	Audrian, 2011	Bux, 1998		Bruin, 1999
technique	MAIGA	GIFT	MAIGA	GIFT
specificity:				
HNA-1a	40/54	74/240	--	16/21
HNA-1b	5/54	7/240	--	2/21
CD16b	9/54	34/240	34/125	1/21
CD11b/CD18	--	--	26/125	
CR1 (CD35)	--	--	18/125	
Fc γ RII (CD32)	--	--	3/125	
none	--	159/240	--	2/21

How about the other specificities?

study	Audrian, 2011	Bux, 1998		Bruin, 1999
technique	MAIGA	GIFT	MAIGA	GIFT
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CD11b/CD18	--	--	26/125	
CR1 (CD35)	--	--	18/125	
Fc γ RII (CD32)	--	--	3/125	
none	--	159/240	--	2/21

CD11b/CD18

But ...

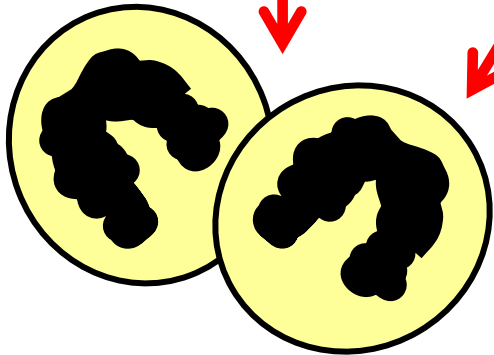
... after all, there is **no correlation** between antibody levels and neutropenia

... and **no correlation** between neutropenia and infections

Why do ab levels and neutropenia lack association?

variation in phagocytosis

[Hadley *et al*, Br J Haematol 1986; Hadley *et al*, Br J Haematol 1987]



complement activation

IgG-dependent C3 deposition
with sera from Felty patients

[Rustagi *et al*, J Clin Invest 1982]

30/60 AIN activated complement

[Bux *et al*, Blood 1998]

anti-precursor activity

maturational arrest, hypoplasia
detected in some patients

*not consistent with ab findings**

[Harmon *et al*, Br J Haematol 1984; Currie *et al*, Blood 1987;

* Hartman *et al*, Blood 1994]

reduced neutrophil apoptosis

more pronounced for NA1 cells
with SLE, SS, RA sera

[Durand *et al*, J Leukoc Biol 2001]

Why do neutropenia and infection lack association?

adhesion deficiency

in moab studies

[Boxer & Stossel, J Clin Invest 1974]

detected in RA, SS, SLE patients

[Durand *et al*, J Leukoc Biol 2001]

motility disturbance

detected in 1 patient without neutropenia

[Kramer *et al*, New Engl J Med 1980]

depressed ROS production

*detected in 1 Felty patient with autoanti-CD11b/CD18**

[Hartman & Wright, Blood 1991]

and other Felty patients,

[Davis, Ann Rheum Dis 1987; Friman, Med Biol 1984]

in RA, SS, and SLE patients

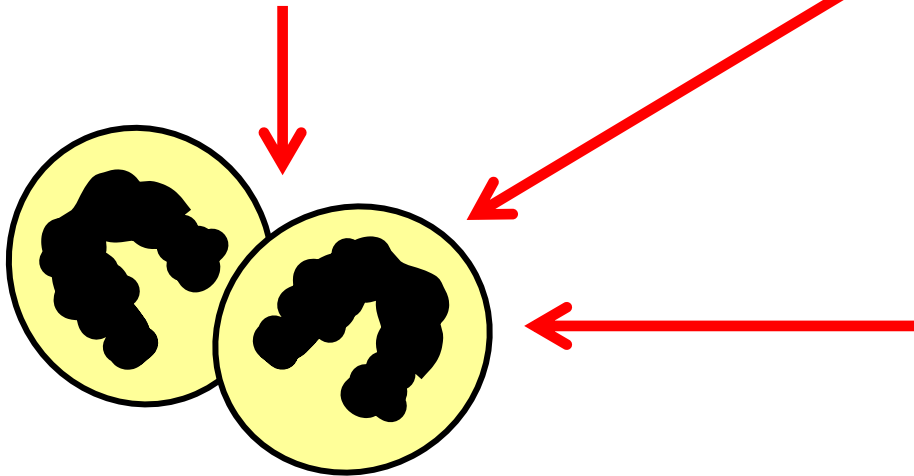
[Durand *et al*, J Leukoc Biol 2001]

and in moab studies

[Martin *et al*, J Leukoc Biol 1984]

*26/125 and 4/14 children with AIN were reported to have anti-CD11b/CD18 autoantibodies

[Bux *et al*, Blood 1998; Bruin *et al*, Blood 1999]



How about the other specificities?

study	Audrian, 2011	Bux, 1998		Bruin, 1999
technique	MAIGA	GIFT	MAIGA	GIFT
specificity:				
HNA-1a	40/54	74/240	--	16/21
HNA-1b	5/54	7/240	--	2/21
CD16b	9/54	34/240	34/125	1/21
CD11b/CD18	--	--	26/125	
CR1 (CD35)	--	--	18/125	
Fc γ RII (CD32)	--	--	3/125	
none	--	159/240	--	2/21

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?



Five different **blood group systems** exist on neutrophils: HNA-1 (on CD16b), HNA-2 (on CD177), HNA-3 (on CTL2), HNA-4 (on CD11b) and HNA-5 (on CD11a).

It is important to know about them when diagnosing **neonatal immune neutropenia (NIN)**. Genotyping is well established and serology testing available.

HNA-1 (anti-HNA-1a?) and anti-CD11b/CD18 seem to have some impact on **autoimmune neutropenia (AIN)**, but how exactly is unclear. It has not yet been unraveled if and how different antibody specificities affect the course of AIN in affected patients.

Because anti-HNA-1a is a frequent (pseudo?)specificity in AIN, search panels should include HNA-1aa homozygous cells (approx. 15%).

Thank you! Further questions?



Foto: Rolf K. Wegst

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