

Inhibitory activity of antibodies evaluated on different type of primary target cells

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Antibodies able to inhibit HIV-1 *in vitro*

- **Neutralizing Abs (NAbs):**

Abs able to inhibit HIV replication *in vitro*

(Ig alone without participation of FcR or C)

- **Non-neutralizing inhibitory Abs**

Mechanisms involving of Fc γ R

- *ADCC: Antibody dependant cellular cytotoxicity => NK*

- *Ab binding to cell free HIV*

Macrophages: Fc γ RI and Dendritic cells: Fc γ RII

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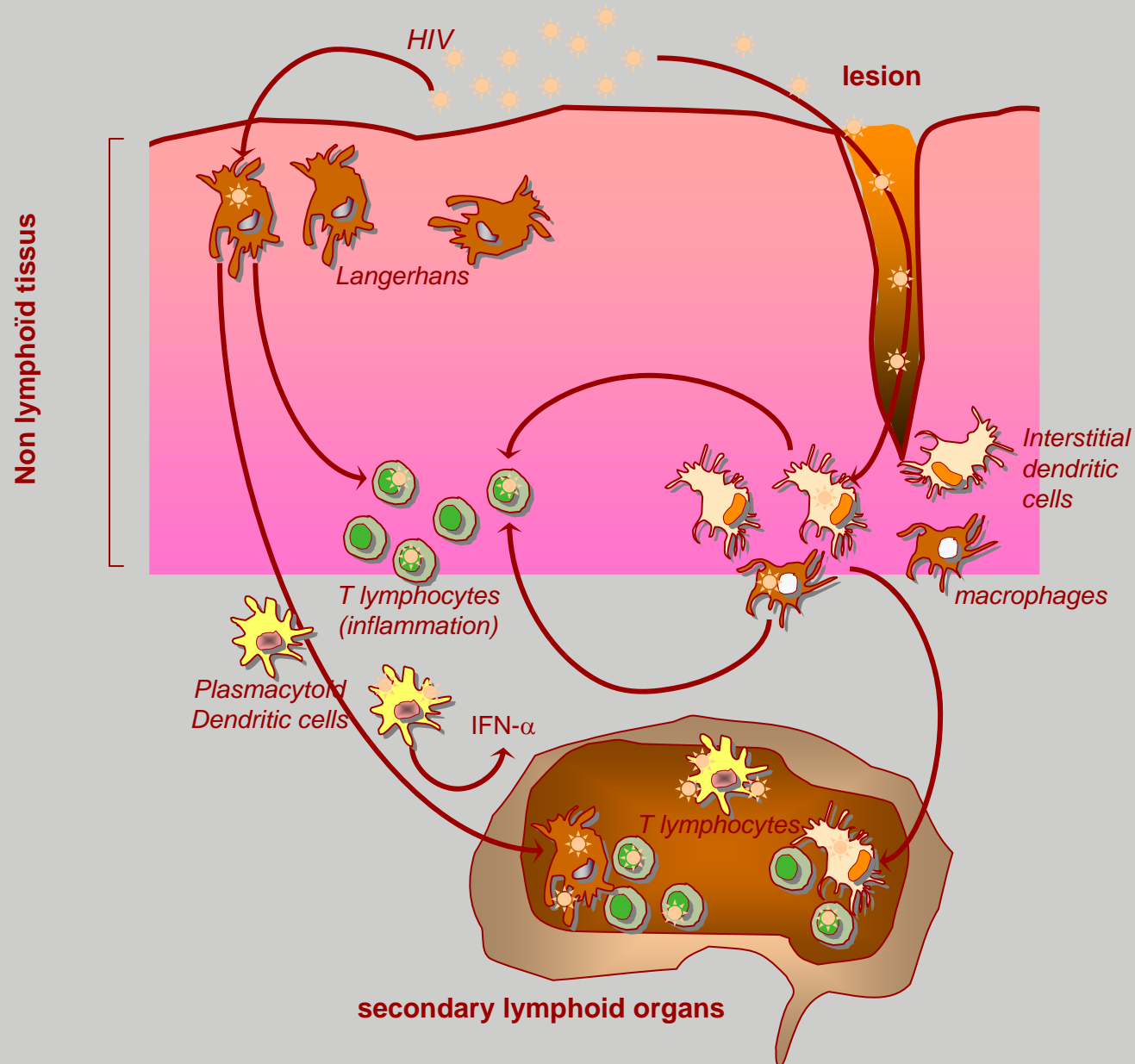
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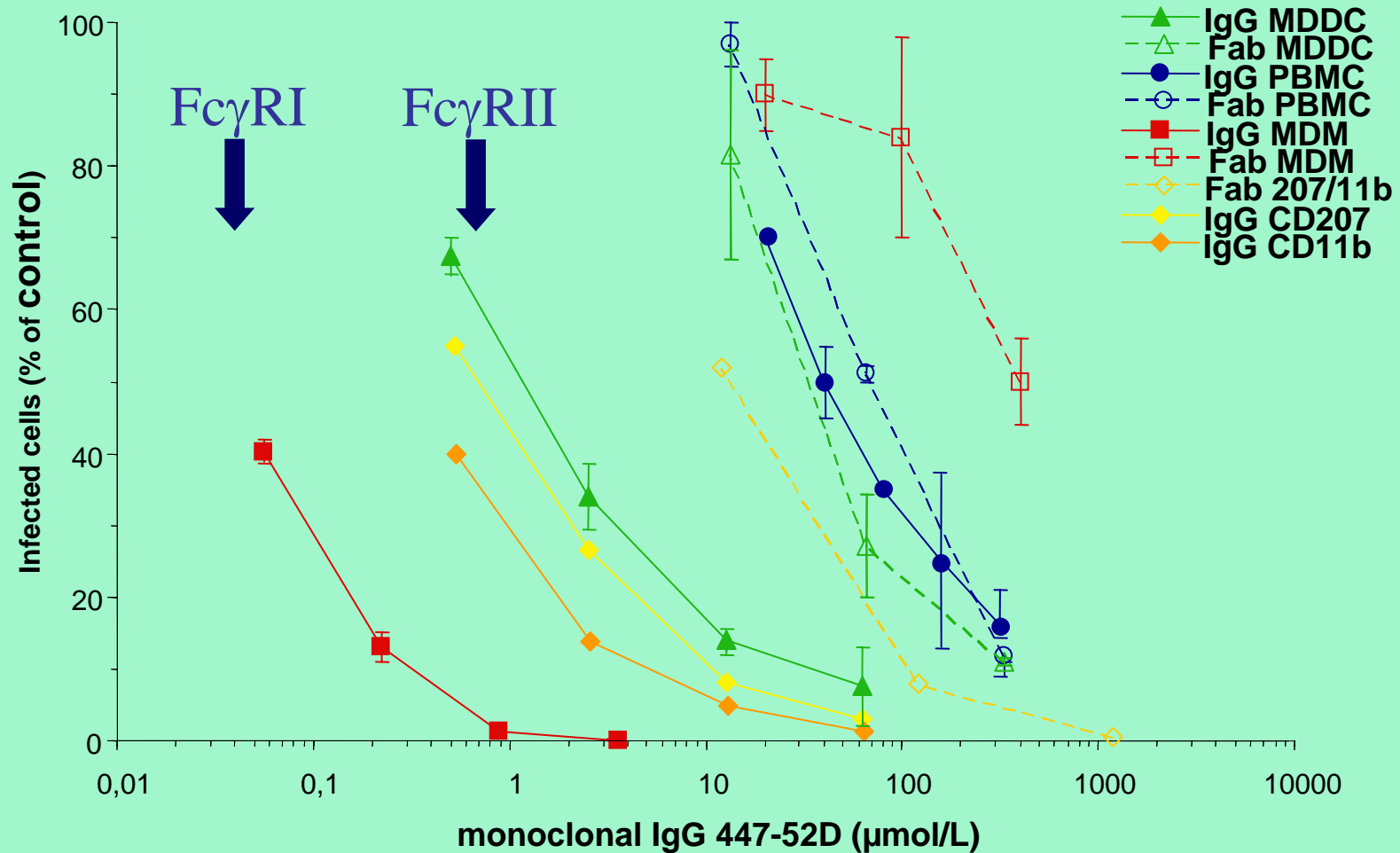
Macrophages: Fc γ RI and Dendritic cells: Fc γ RII

HIV target cells at the mucosal site



Inhibition of HIV by neutralizing mAb 447D

(Collaboration Susan Zolla-Pazner)



Neutralizing MAbs: macrophages > iDC > Lymphocytes

Increased inhibitory activity

=> second mechanism of inhibition that involves FcγR

Neutralizing mAb b12 and mutants in Fc γ R

(Collaboration Dennis Burton)

Ab	Mutant	Passive transfer of Ab Protection of macaques from SHIV162P3 vaginal challenge
b12	IgG WT	8/9 macaques
KA	no binding to C1q	8/9 “
LALA	no binding to Fc γ R and C1q	5/9 “

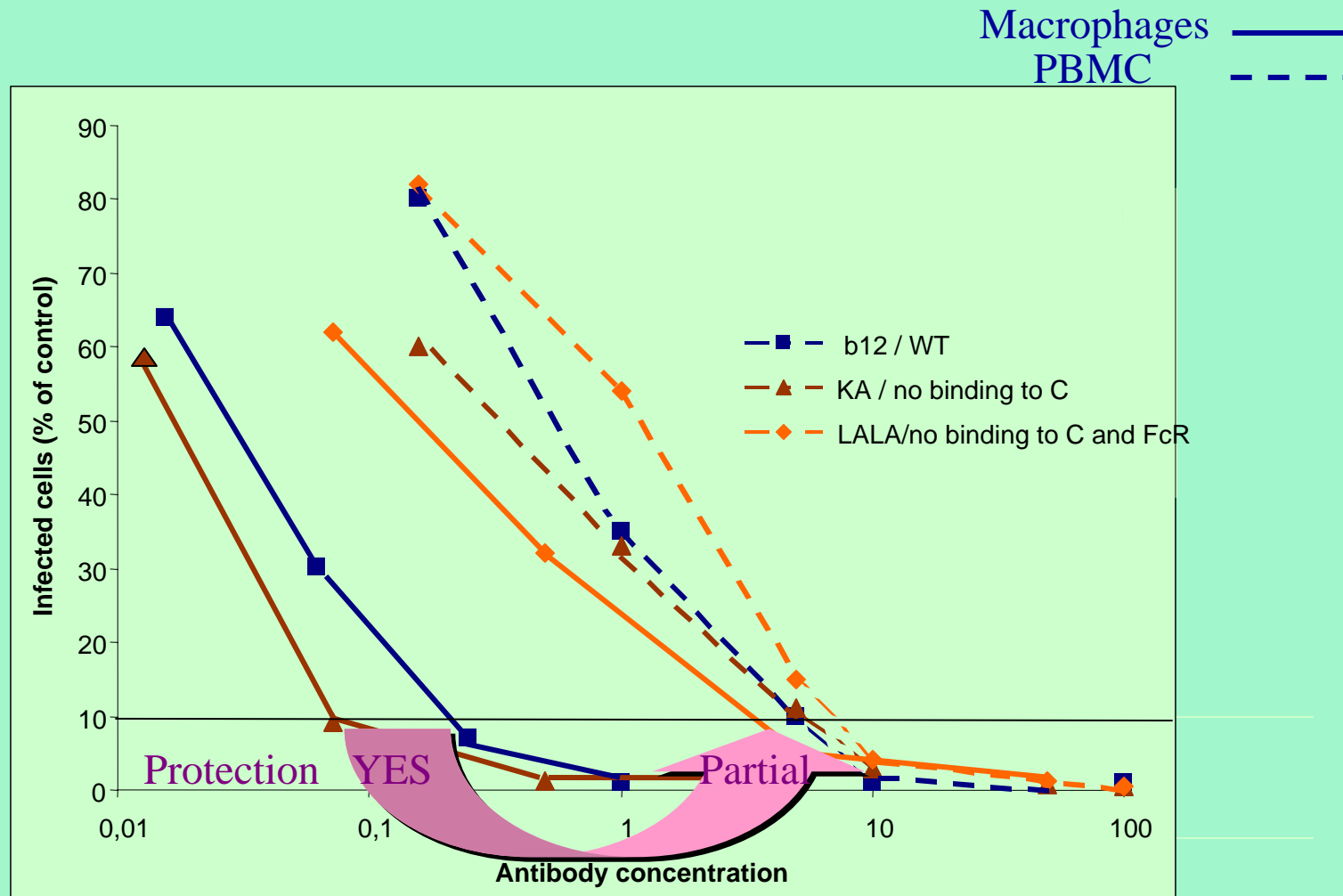
Fc γ part of IgG participates in protection

D. Burton AIDS vaccine 2006 Amsterdam

What is the inhibitory activity on macrophages?

Inhibitory activity of neutralizing mAb b12 and b12 mutated in FcγR

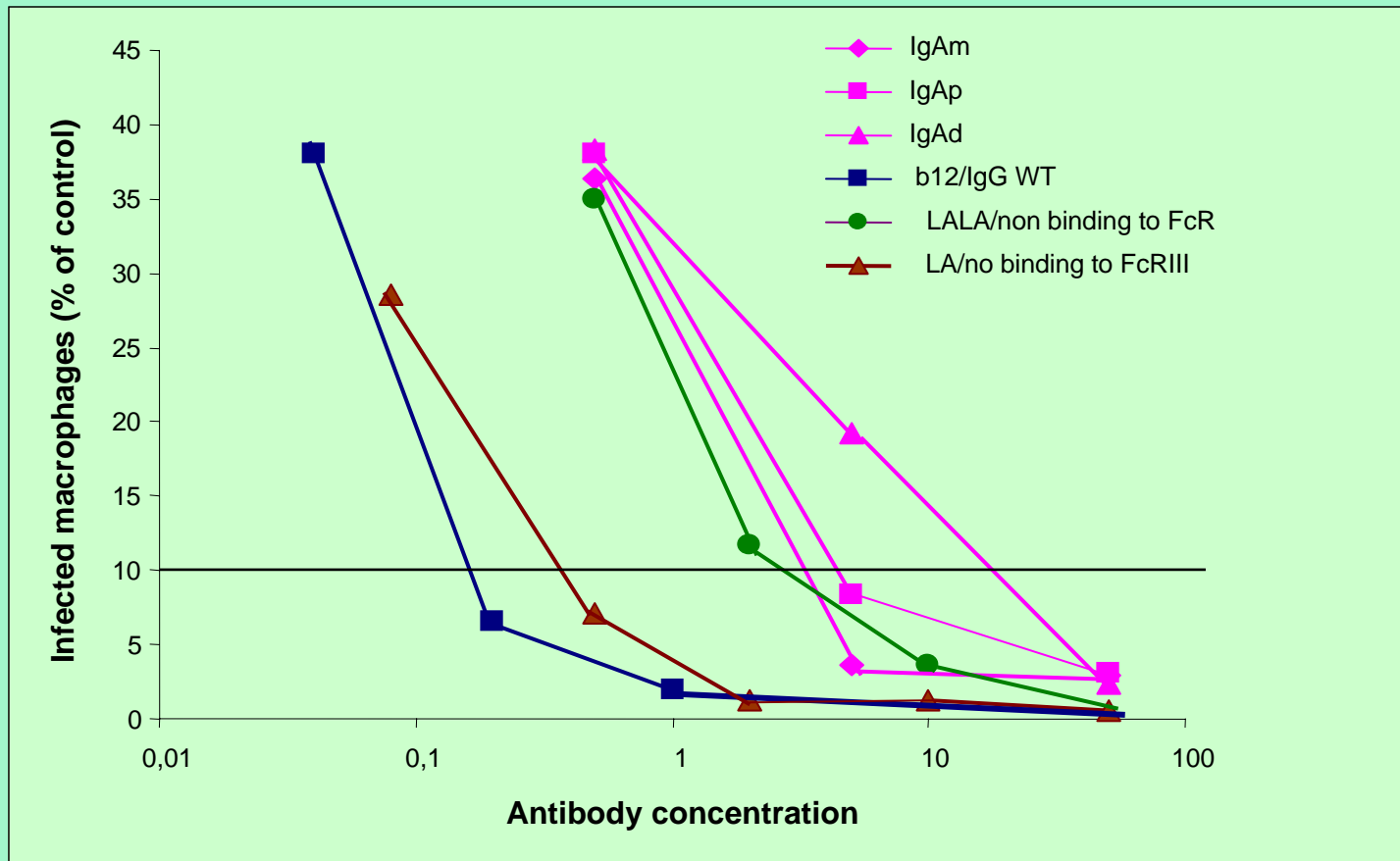
(Collaboration Dennis Burton)



1.5 log

Does this mechanism involving FcγR play a role in protection against vaginal infection?

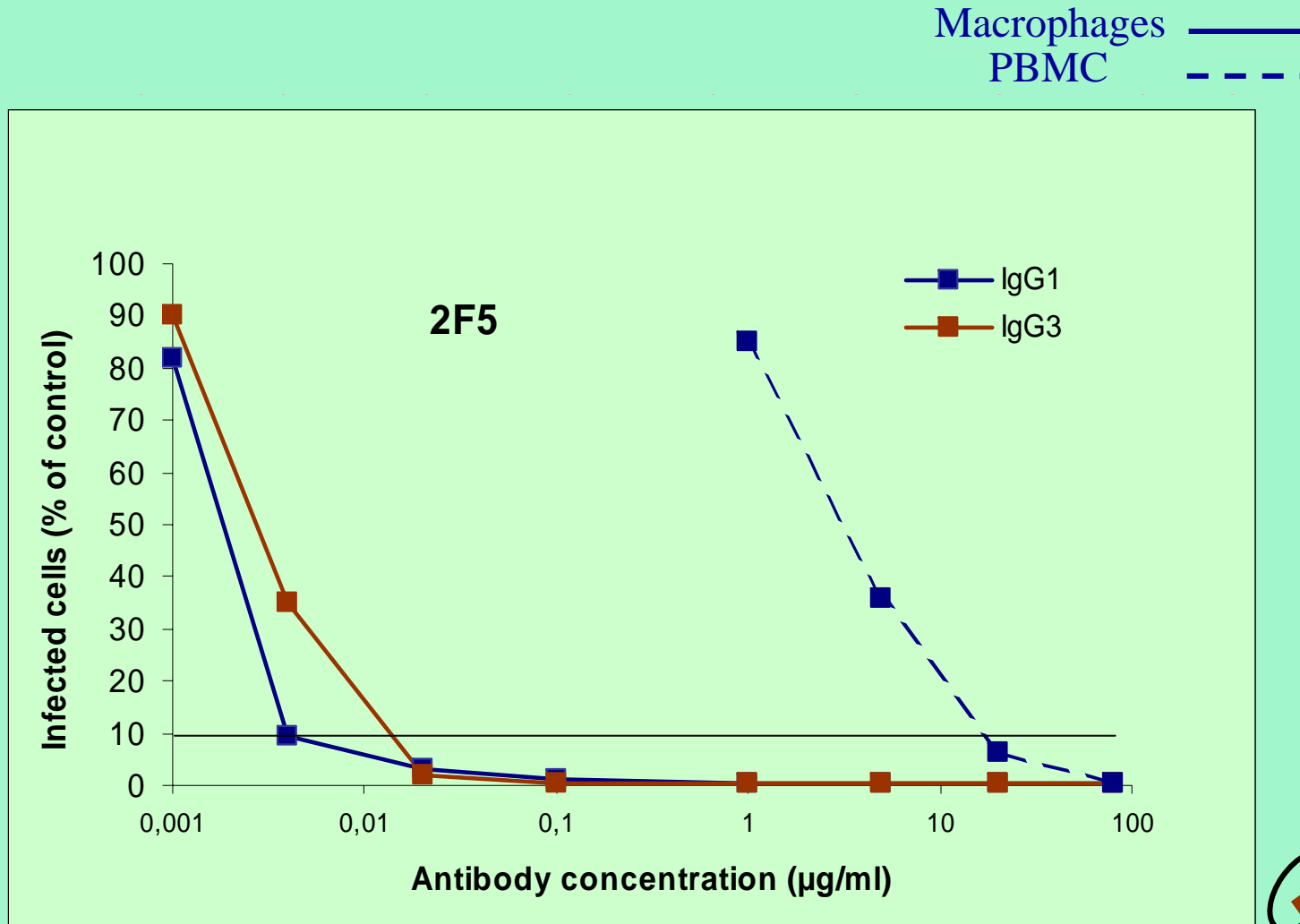
Inhibitory activity of Ig type of neutralizing mAb b12 in macrophages (Collaboration Dennis Burton)



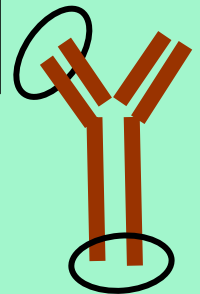
Second mechanism of inhibition involves Fc γ RI on macrophages

IgG sub-types of neutralizing mAb 2F5

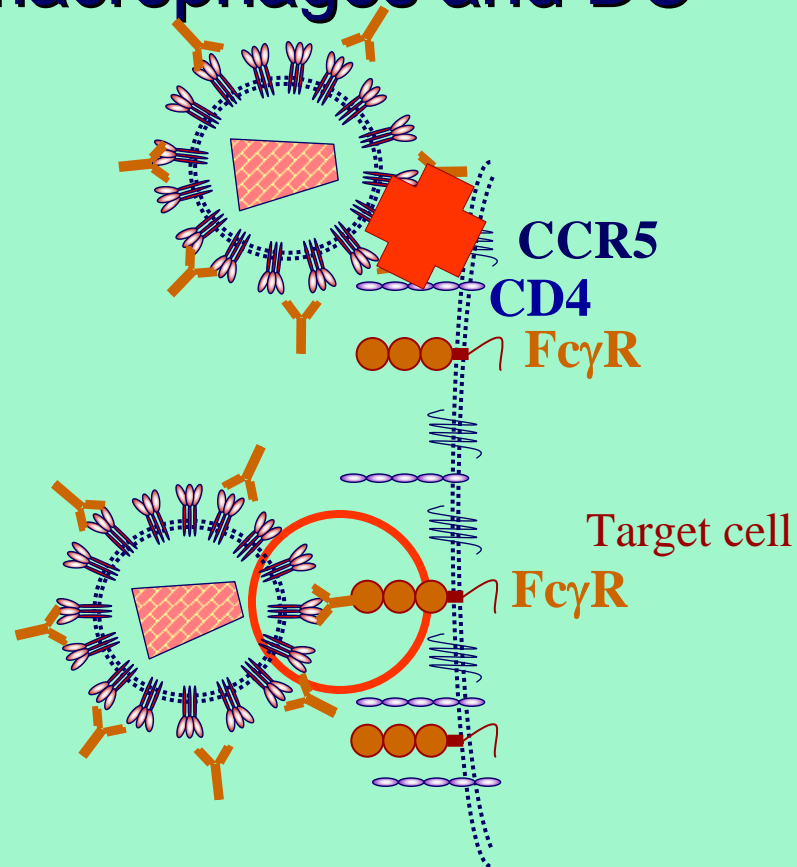
(Collaboration R. Kunert, Polymun Scientific GmbH)



Second mechanism: subtype IgG1 > IgG3
role of the heavy chain of IgG1 ?



Mechanism of HIV inhibition by NAbs on macrophages and DC



- Neutralization of infectivity via Fab part of Ig
Common for T lymphocytes T, DC et macrophages
- **Inhibition of infection *via* FcγR I (macrophages) or II (DC)**

Question 1

**Are non-neutralizing antibodies
able to inhibit
with this 2nd mechanism of inhibition?**

YES

Inhibitory activity of MAbs able to bind to native envelope

11-100 µg/ml
1-10 µg/ml
0.0025-0.5µg/ml

Ref. N°	Name			Epitope	CD4 T cells	Macrophages
ARP 301	221	IgG1	gp 120	gp160/ gp120 (aa 482-495) C term of gp120	>50	20
NIH 857	F105	IgG1k		conformational gp120	>100	>50
NIH 7369	654-30D	IgG1l		tertiary gp120	nd	Ni
ARP 3119	CA13	IgG1		cross reactive to env	nd	Ni
ARP 390	ICR39.13	IgG2b		conformational (gp120)	Ni	Ni
ARP 3041	11/68b	IgG1		gp120 (V1, V2+C4)	Ni	Ni
ARP 3036	8/64b	IgM	V3 loop	V3 (aa 300-315)	nd	Ni
ARP 3038	10/540.w	IgG1		V3 (aa 311-321)	Ni	Ni
ARP 3039	8/38	IgG2a		V3 (aa 300-315)	nd	Ni
EVA 331	178.1.1	IgG2ak		V3 (to KSIRI sequence)	Ni	Ni
EVA 3047	IIIB-V3-13	IgG1		V3 (IRIQRGPGGRAFTIGC sequence)	>50	3
ARP 3023	257-D IV	IgG1λ		V3 (KRIHI sequence)	>9.4	0.05
ARP 3024	268-D IV	IgG1λ		V3 (HIGPGR sequence)	>14	1
EVA 3056	MN215	IgG1		V3 KS/GIHIGPGKAFYTTGEI sequence)	>125	10
NIH 7625	F425B4a1	IgG1λ		V3	>33	0.4
	391-D	IgG1k		V3	20	1
NIH 7626	F425B4e8	IgG1k		base of V3 loop	25	1
NIH 2534	4G10	IgG1	V3 (RIQRGPGRAFVTGK)	nd	Ni	
ARP 324	CRA3	IgG2a	V2	conformational (V2 and C1)	Ni	Ni
ARP 325	CRA4	IgG1		conformational (V2)	Ni	Ni
ARP 3075	62c	IgG1		conformational V2	Ni	Ni
ARP 3218	697D	IgG1λ		V2 (conformational, region 164-194)	>43	>20
	847-30	IgG1λ	C2,4,5	C2	nd	>50
ARP 388	ICR38	IgG2b		C4 (aa427-436)	Ni	Ni
	858-D	IgG3λ		C5 (aa 495-516)	>100	>50
	1331A	IgG3λ		C5 (aa 495-516)	>100	>50
	450-D	IgG1λ		C5 (aa 503-509)	>100	>50
	722-D	IgG1k		C5 (aa 503-509)	nd	>50
	670D	IgG1λ		C5 (aa 503-509)	>100	>50
EVA 3055	GP68	IgG1	CD4 bs	CD4 binding site	Ni	Ni
ARP 3220	654D	IgG1λ		CD4binding site (discontinuous)	>25	>20
	570-D	IgG1λ		CD4binding site	>100	>50
	654-D	IgG1λ		CD4binding site	>100	>50
ARP 3078	1.7B	IgG1		CD4 induced	>50	>50
ARP 3079	4.8D	IgG1		CD4 induced	>50	>50
NIH 6882	5F3	IgG1λ	gp 41	gp41 (526-543)	nd	>100
	181-D	IgG1k		gp141 (I)	>100	>50
	240-D	IgG1k		gp41 (aa 579-604) (I)	>35	1.8
	246-D	IgG1k		gp41 (aa 579-604) (I)	>100	0.8
	50-69	IgG1λ		gp41 (aa579-613 conformational) (I)	>100	0.4
NIH 7623	F240	IgG1k		gp41 (aa 592-606)	>100	0.5
	98-6D	IgG1k		gp41 (aa 644-663) (II)	>100	>50
	126-6	IgG1k		gp41 (aa 644-663) (II)	>100	>50
	167-D	IgG1λ		gp41 (aa 644-663) (II)	>100	>50
NIH 1172	1577	IgG3		gp41 (731-752)	nd	25

Non-neutralizing antibodies inhibit HIV infection on macrophages or dendritic cells

Name	Epitope		IC90 (µg/ml)			iDC	CD4 T Cells		
			Macrophages				BaL	BaL	Bx08
			BaL	Bx08	TV1				
221	gp160/ gp120 (aa 482-495)	gp120	20	50	>100	nd	>50	nd	
IIIB-V3-13	V3 (IRIQRGPGRAFTIGC)	V3 loop	3	nd	nd	nd	>50	nd	
257-D IV	V3 (KRIHI sequence)		0.05	0.3	>9	10	>9.4	nd	
268-D IV	V3 (HIGPGR sequence)		1	0.5	>15	15	>14	>14	
MN215	V3 (KS/GIHIGPGKAFYTTGEI)		10	40	20	>50	>125	>125	
391-D	V3		1	0.8	20	nd	20	nd	
F425B4a1	V3		0.4	nd	nd	nd	>33	nd	
F425B4e8	base of V3 loop		1	5	>30	4	25	nd	
240-D	PID gp41 (aa 579-604) (I)	gp41	1.8	1.9	0.5	20	>35	>35	
246-D			0.8	0.5	0.06	45	>100	>100	
50-69			gp41 (aa579-613 conformational)	0.4	0.8	0.6	>50	>100	>100
F240			gp41 (aa 592-606)	0.5	0.6	15	50	>100	nd
1577			gp41 (731-752)	25	100	nd	>100	nd	nd

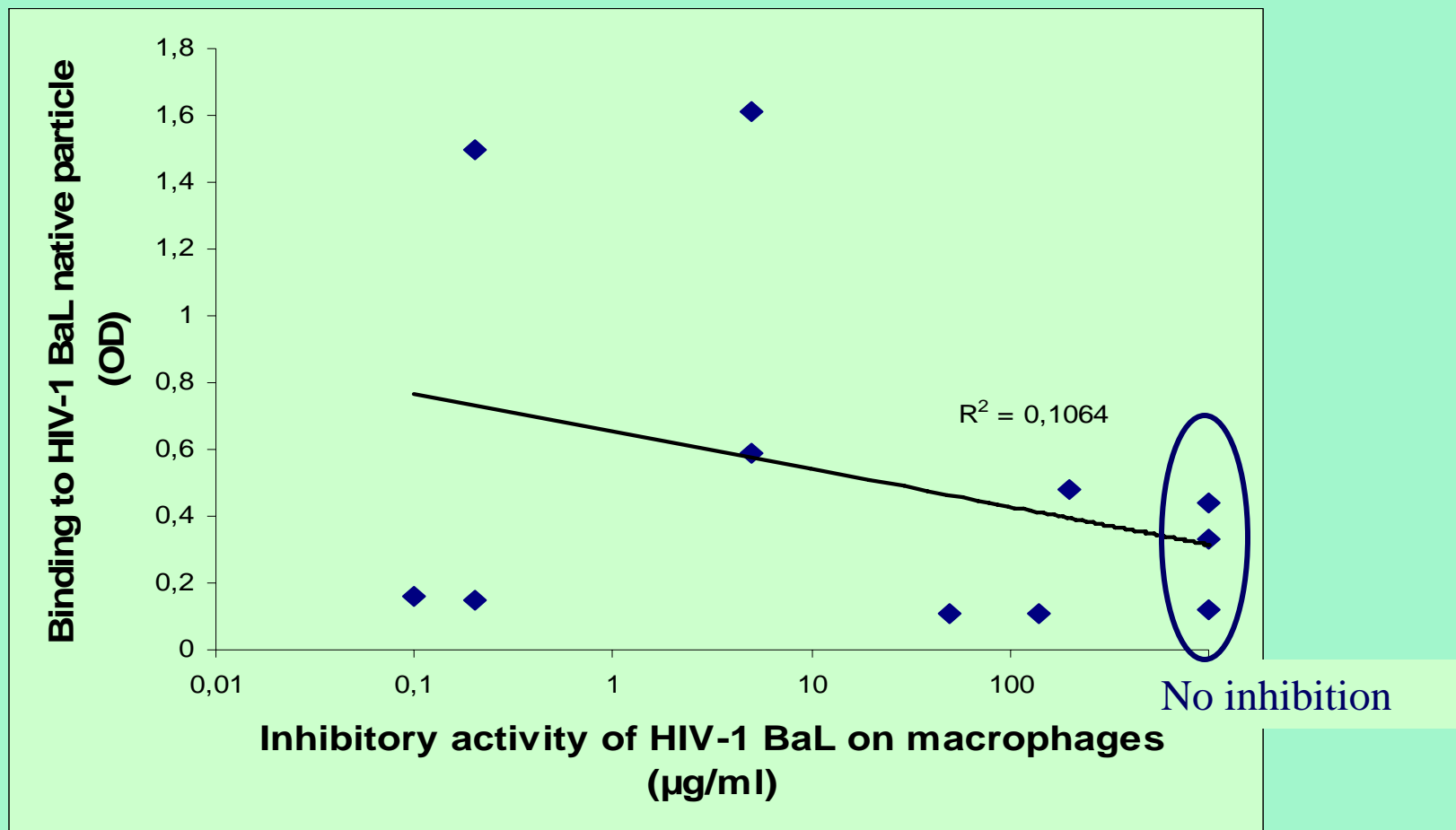
Inhibitory activity on macrophages of other non neutralizing mAbs (R. Kunert, Polymun Scientific GmbH)

Name	epitope	Sequence	<i>Macrophages</i>	
			BaL	TV1
3D5	gp120	ND	140	>140
1B1		ND	50	>50
2G6		ND	>200	>100
1F7		ND	0.1	>100
1G7		ND	0.2	>100
4D4	PID gp41	579-613	5	10
4B3		579-613 (GCSGKLICTTAVPW)	0.2	0.2
25C2		526-543	5	>50
3B7		ND	200	100
6F8		ND	>100	>100
3H12		ND	>100	>100

11-100 µg/ml
5-10 µg/ml
1-0.3 µg/ml
0.2-0.05 µg/ml

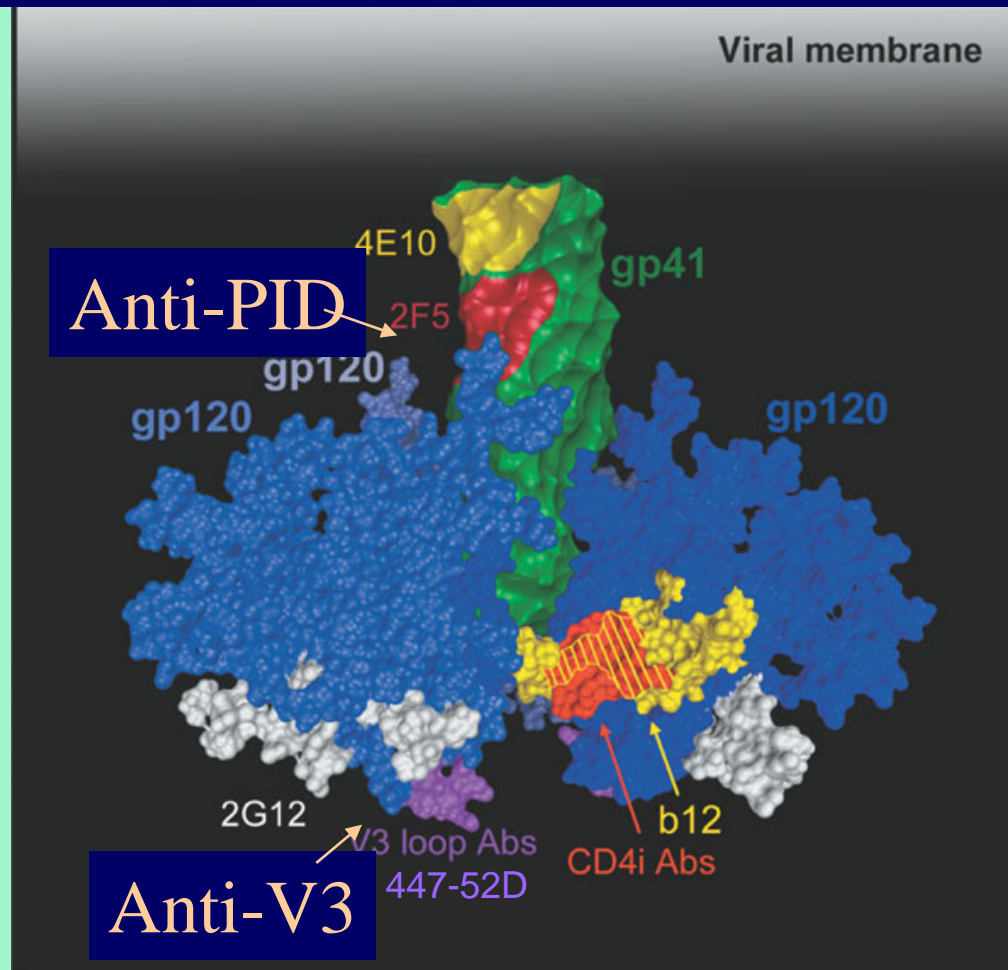
ND: not determined

No correlation between inhibitory activity on macrophages and binding to native particle (R. Kunert, Polymun Scientific GmbH)



Epitopes recognized by the 5 Neutralizing Mab

«Non-neutralizing» inhibitory MAbs



Additional epitopes to target for inhibition of HIV activity

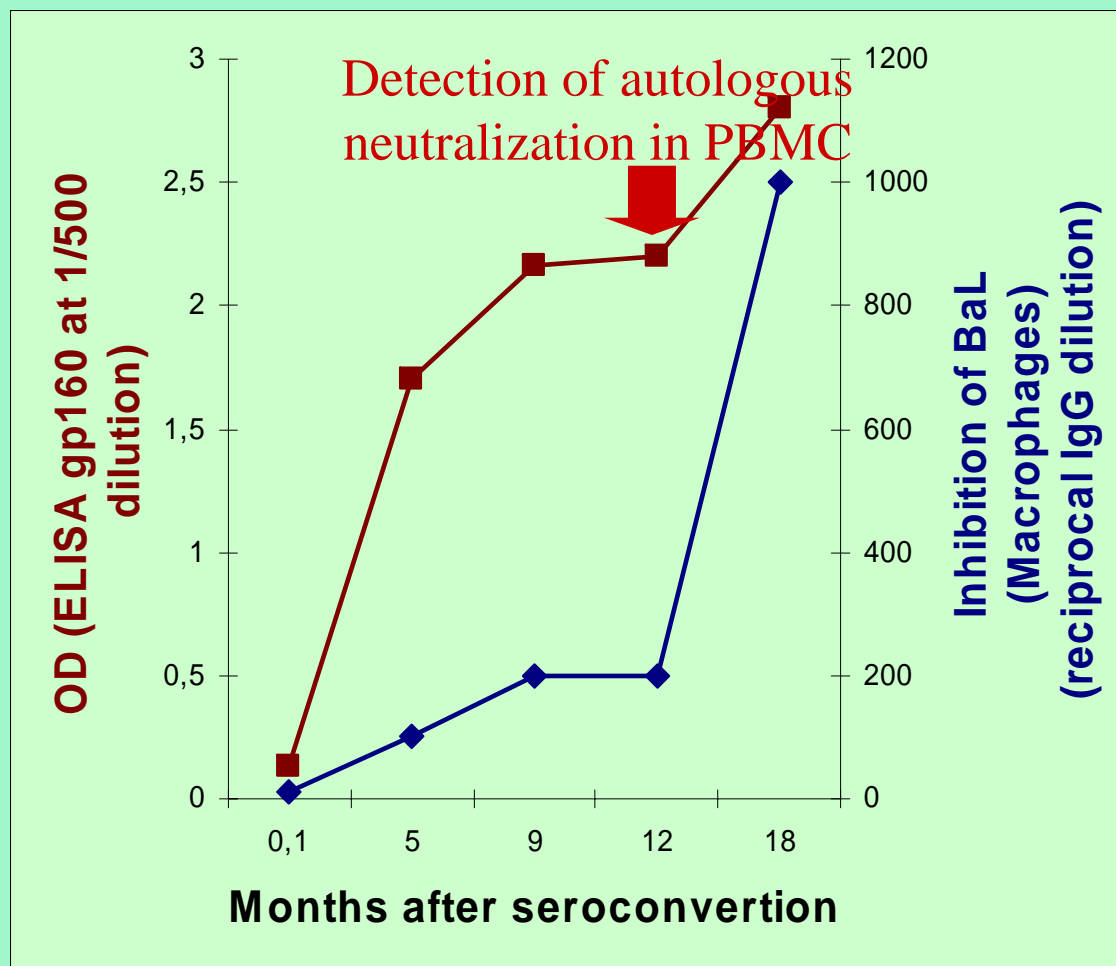
Question 2

Are

non-neutralizing inhibitory antibodies
present in sera from
infected individuals

YES

Antibodies detection following sero-conversion



Low inhibitory activity on macrophages detected early after infection

Maturation of the non-neutralizing inhibitory activity as for neutralizing Abs ?

Inhibitory activity of IgG from sera of infected individuals when PBMC, Dendritic cells or macrophages are the targets

IgG samples	Neutralizing Titer		
	PBMC	Dendritic cells 1/Dilution	Macrophages
8	1/80	1/400	1/5000
44	1/70	1/330	1/5000
36	1/10	ND	1/1000
24	1/15	1/100	1/300
12	>1/10	ND	1/100
11	>1/10	1/80	1/1500
3	>1/10	1/40	1/2000
HIV negative	>1/10	>1/10	>1/10

Increased inhibitory activity : macrophages > iDC > Lymphocytes

=> non-neutralizing inhibitory Abs frequently induced

Question 3

Are

non-neutralizing inhibitory antibodies

present in sera from

vaccinated volunteers

Are non-neutralizing inhibitory Abs induced by vaccination?

Phase III Vaxgen (Coll. M. Gurwith F. Sinangil, M. Petersen, P. Gilbert.)

- no protection
- non significant trends towards efficacy in preventing infection in the highest risk subgroup



- 53 volunteers from the high risk group (placebo and vaccinated)
- at least 4 injections, 2 weeks post-injection
- uninfected controls matched across timepoints (number of injections)

=> analysis of the inhibitory activity on macrophages

Inhibition of virus BaL by Vaxgen sera in macrophages

	Vaccine treated	Serum	Inhibitory Titer		
			90% reduction	70% reduction	
M64-003	Infected	003	-	15	
M64-004		004	6	20	
M64-008		008	6	20	
M64-012		012	6	20	
M64-016		016	-	15	
M64-023		023	6	20	
M64-025		025	-	10	
M64-028		028	-	10	
M64-031		031	6	30	
M64-034		034	-	-	
M64-041		041	-	-	
M64-044		044	-	-	
M64-045		045	6	20	
M64-047		047	-	10	
M64-051		051	-	-	
M64-053		053	-	15	
M64-006		Non-infected	006	6	15
M64-009			009	-	15
M64-011	011		6	20	
M64-013	013		-	15	
M64-014	014		-	15	
M64-018	018		-	15	
M64-020	020		-	10	
M64-021	021		-	6	
M64-027	027		-	10	
M64-032	032		-	-	
M64-036	036		-	15	
M64-039	039		-	15	
M64-042	042		10	40	
M64-046	046		-	15	
M64-049	049		10	50	
M64-050	050		-	-	
M64-052	052		6	25	

Very low inhibitory activity observed in sera => at the mucosal site?

Question 3

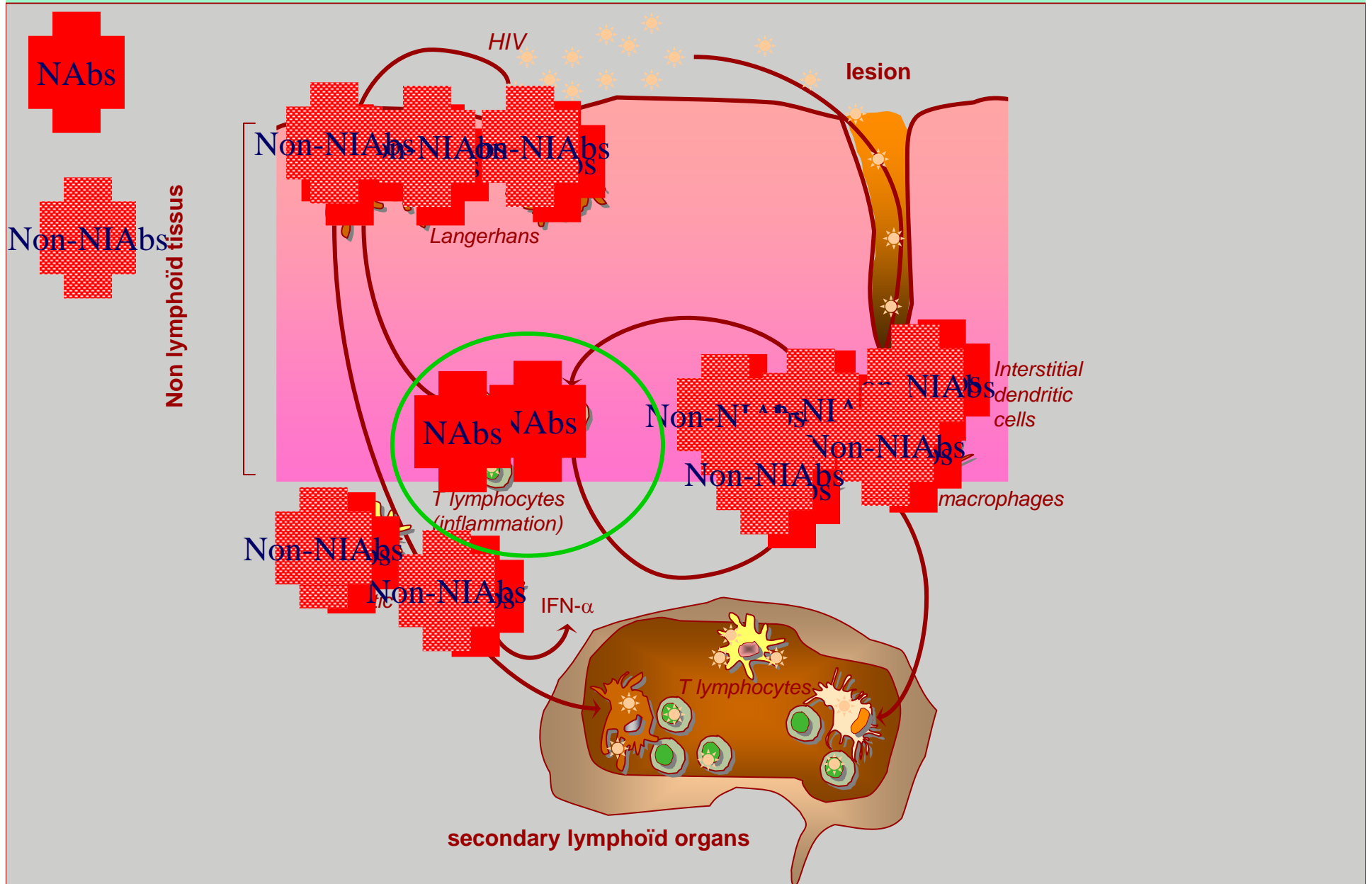
Are

non-neutralizing inhibitory antibodies

**present in sera from
vaccinated volunteers**

Difficult to detect ?

Inhibitory activity of Abs on HIV mucosal target cells



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