Immunogenicity of a trivalent subunit vaccine for genital herpes in Rhesus macaques.

Sita Awasthi, PhD

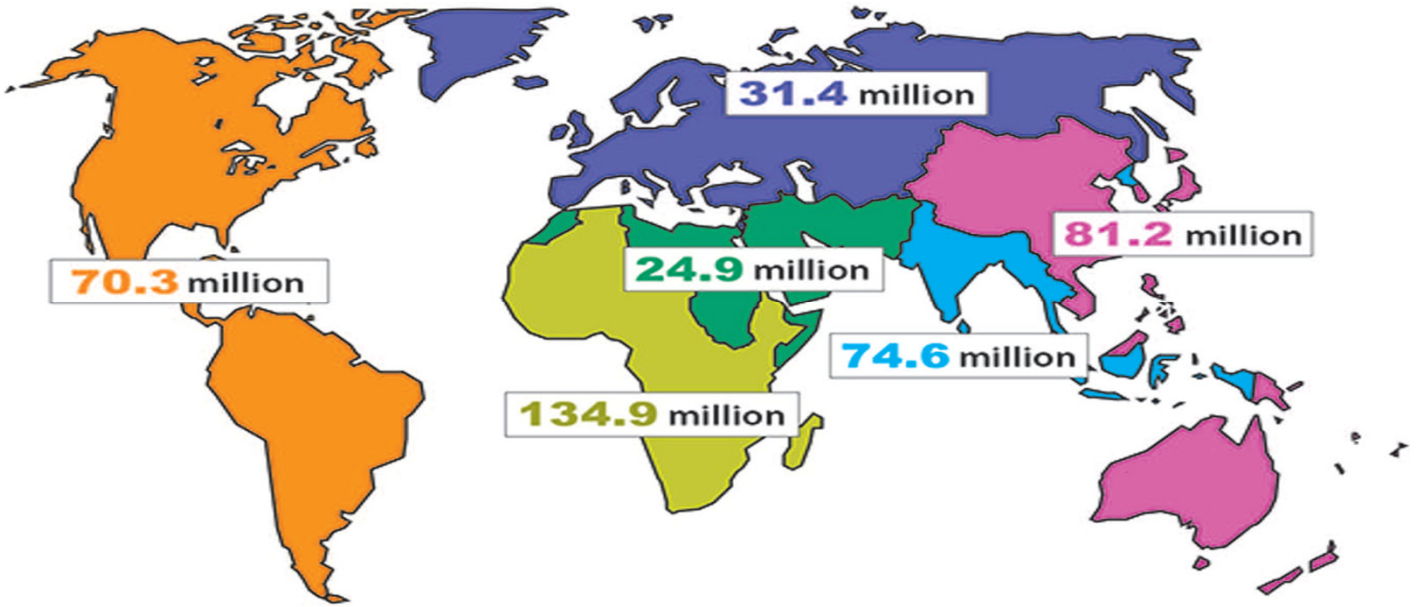
World Congress on Virology, 2015

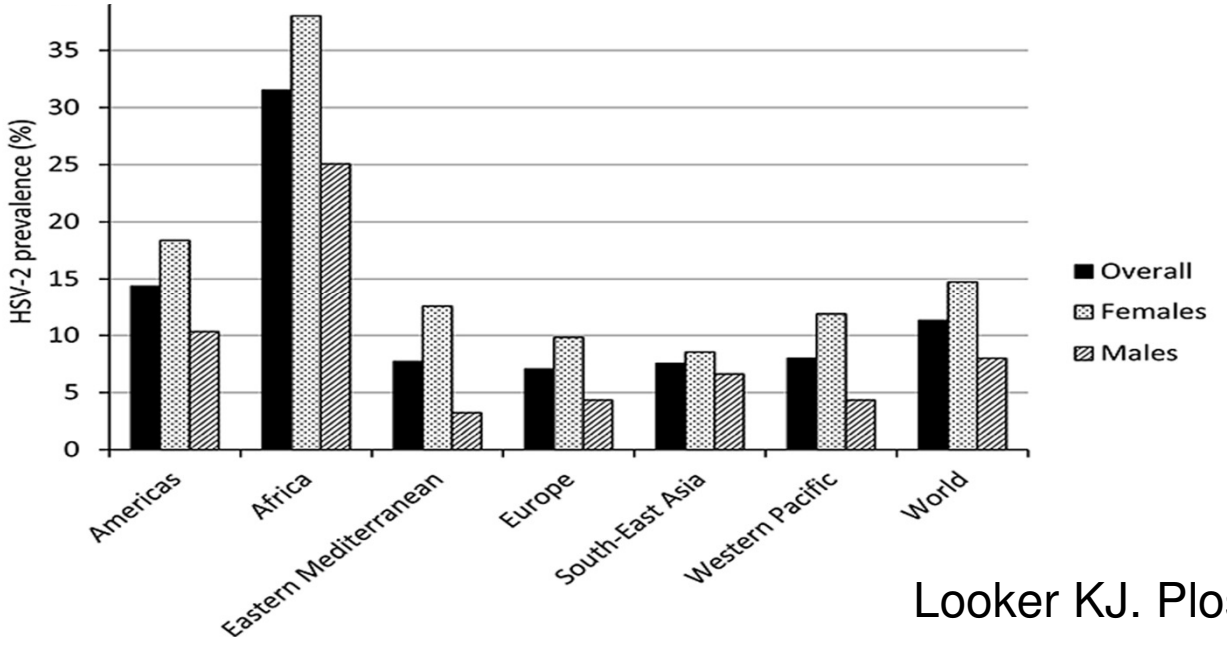
Atlanta, December 7-9





Global prevalence of HSV-2 Infection





Looker KJ. Plos One Jan. 2015

Rationale for HSV-2 vaccine

- Prevention of genital herpes

  15% of adult population is infected with HSV-2 worldwide

  20 million new infections are added each year globally

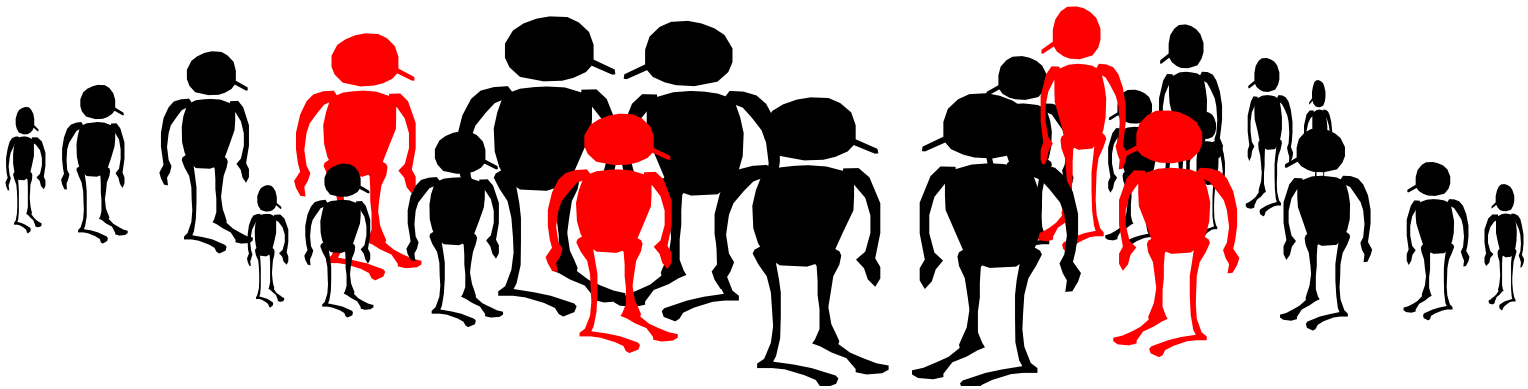
- Treatment of genital herpes

  In US ~ 50 million people are HSV-2 positive

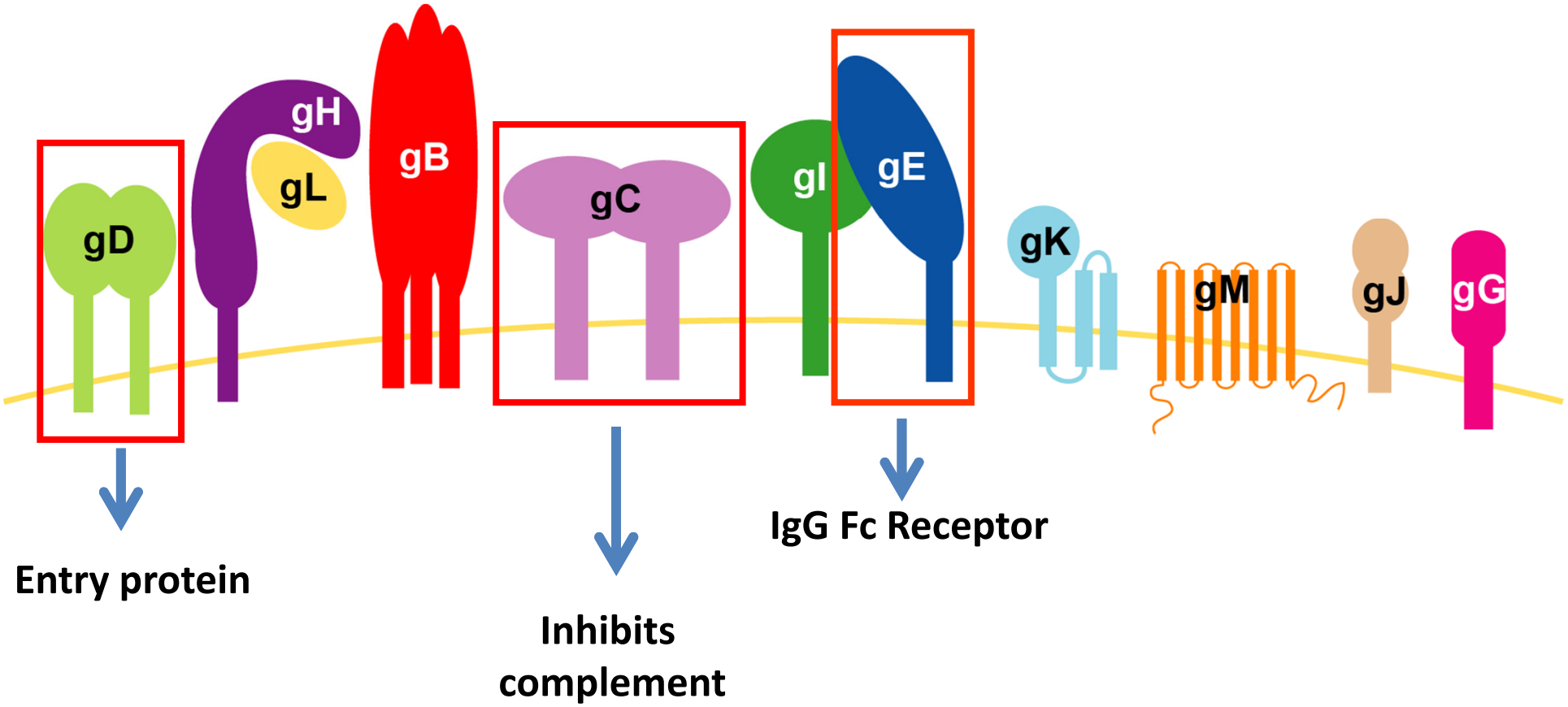
  Economic burden of treatment is ~billion dollars annually in US

- Curb acquisition and transmission of HIV

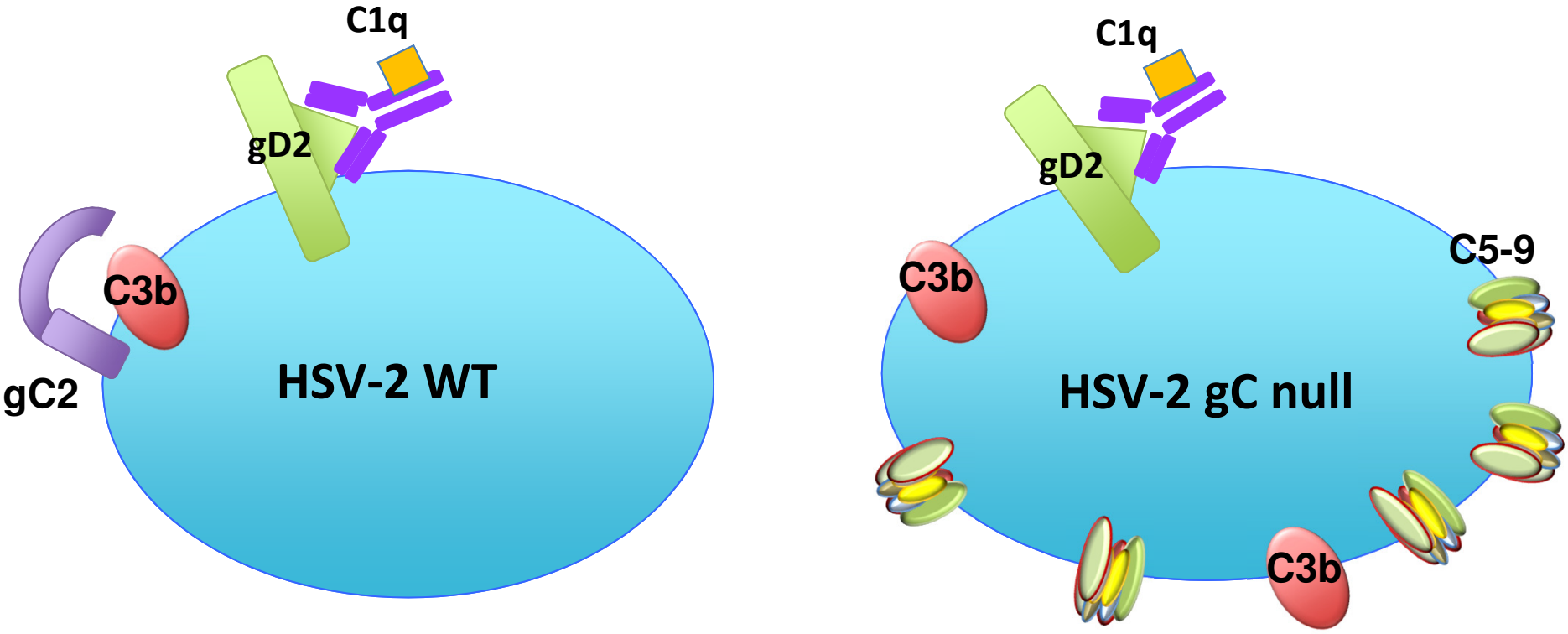
- There is no cure or FDA approved vaccine



Rationale for a trivalent genital herpes vaccine

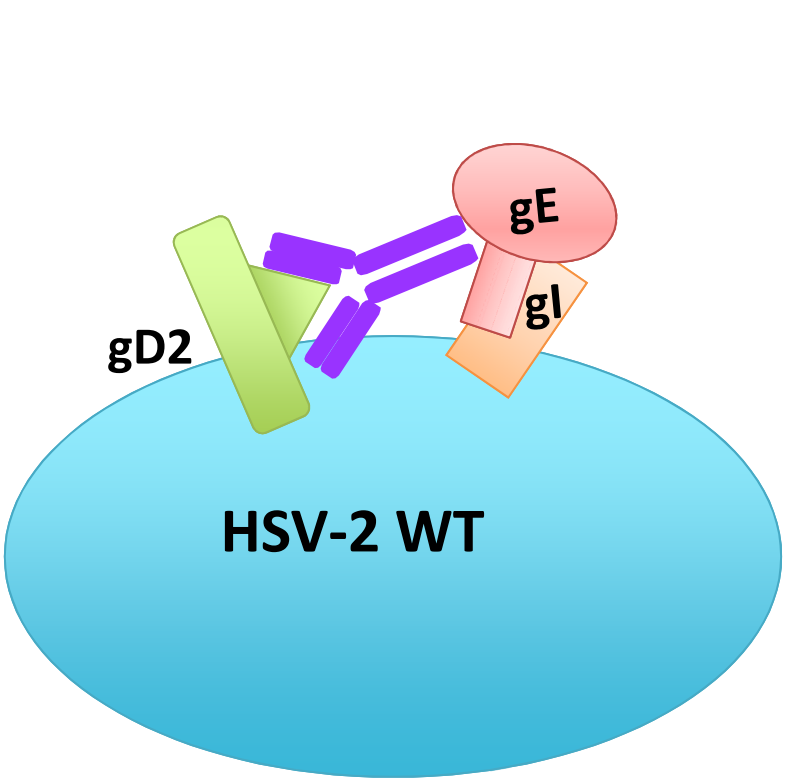


Glycoprotein C protects virus against human complement

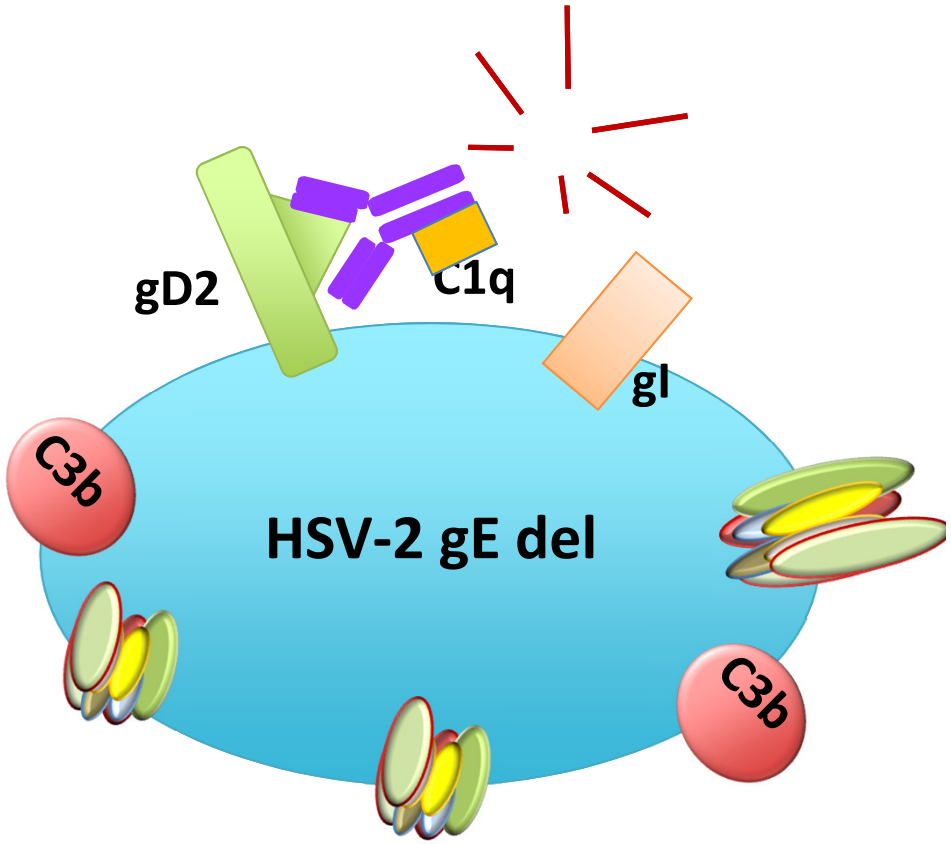


Awasthi et al J Virol 2011

Glycoprotein E protects virus from IgG Fc mediated ADCC and C'activation



WT virus: Antibody bipolar bridging



HSV-2 gE mutant: No antibody bridging

Awasthi et al J Virol 2014

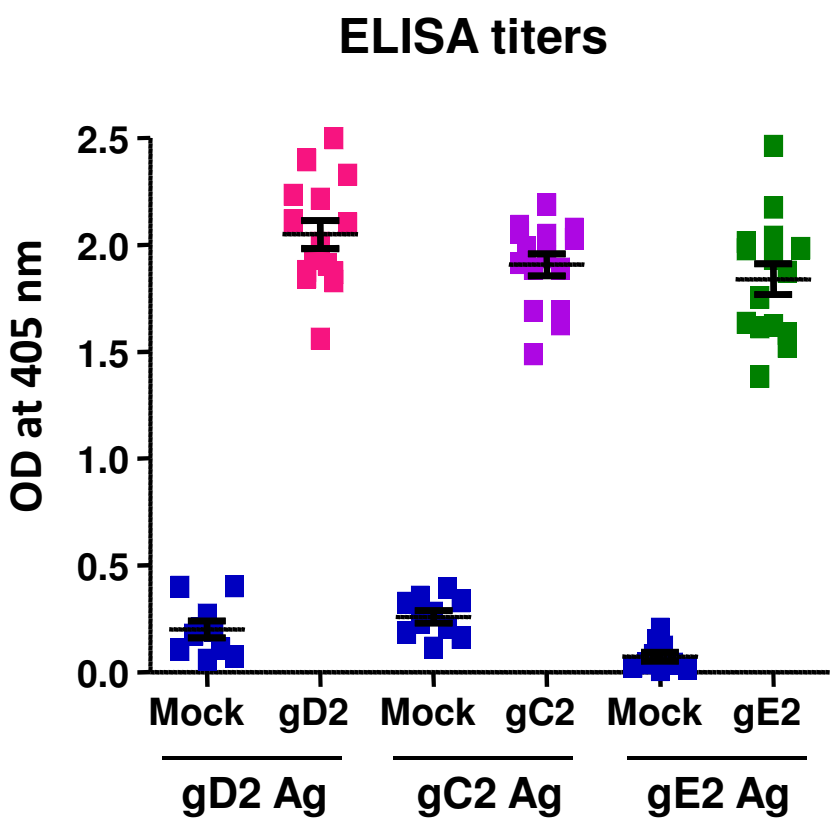
gC2/gD2/gE2 as a prophylactic vaccine

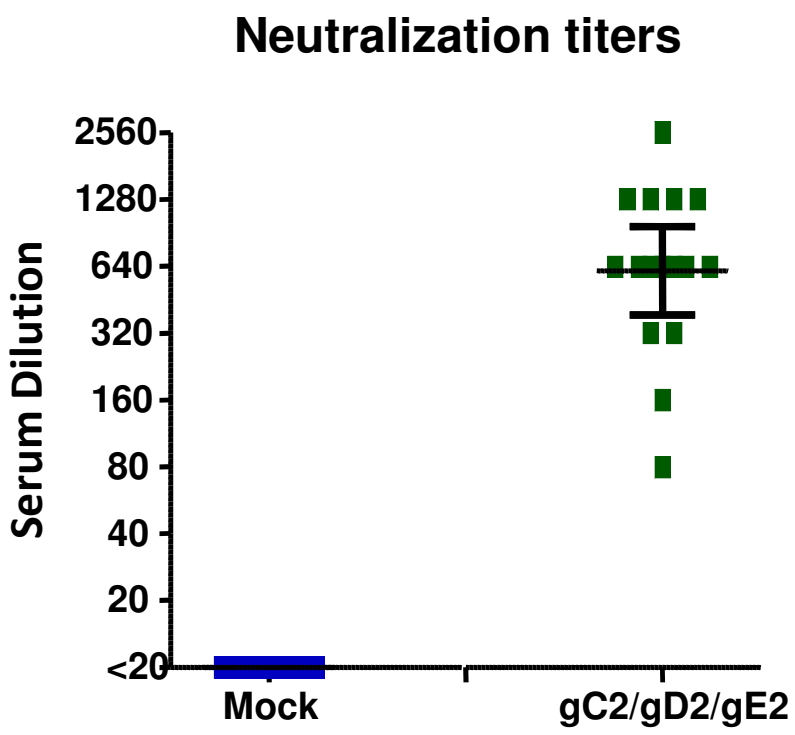
Goal: Elimination of acute and recurrent disease, and asymptomatic shedding of HSV-2 DNA in pre-clinical model.

0-acute
0-recurrent
0-shedding of HSV-2 DNA

Immunogenicity studies: ELISA responses and neutralizing Ab titers





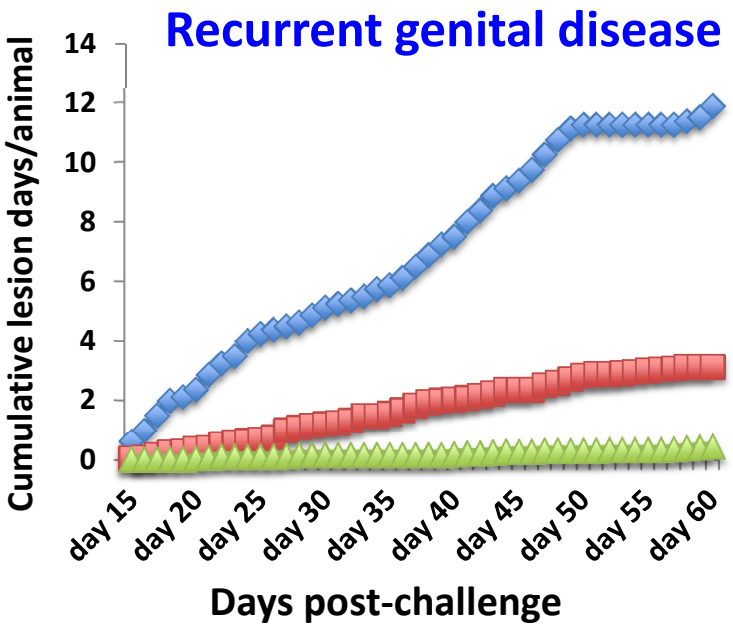


→ High ELISA titers to all immunogens and neutralizing titers to HSV-2

gC2/gD2/gE2 as a prophylactic vaccine

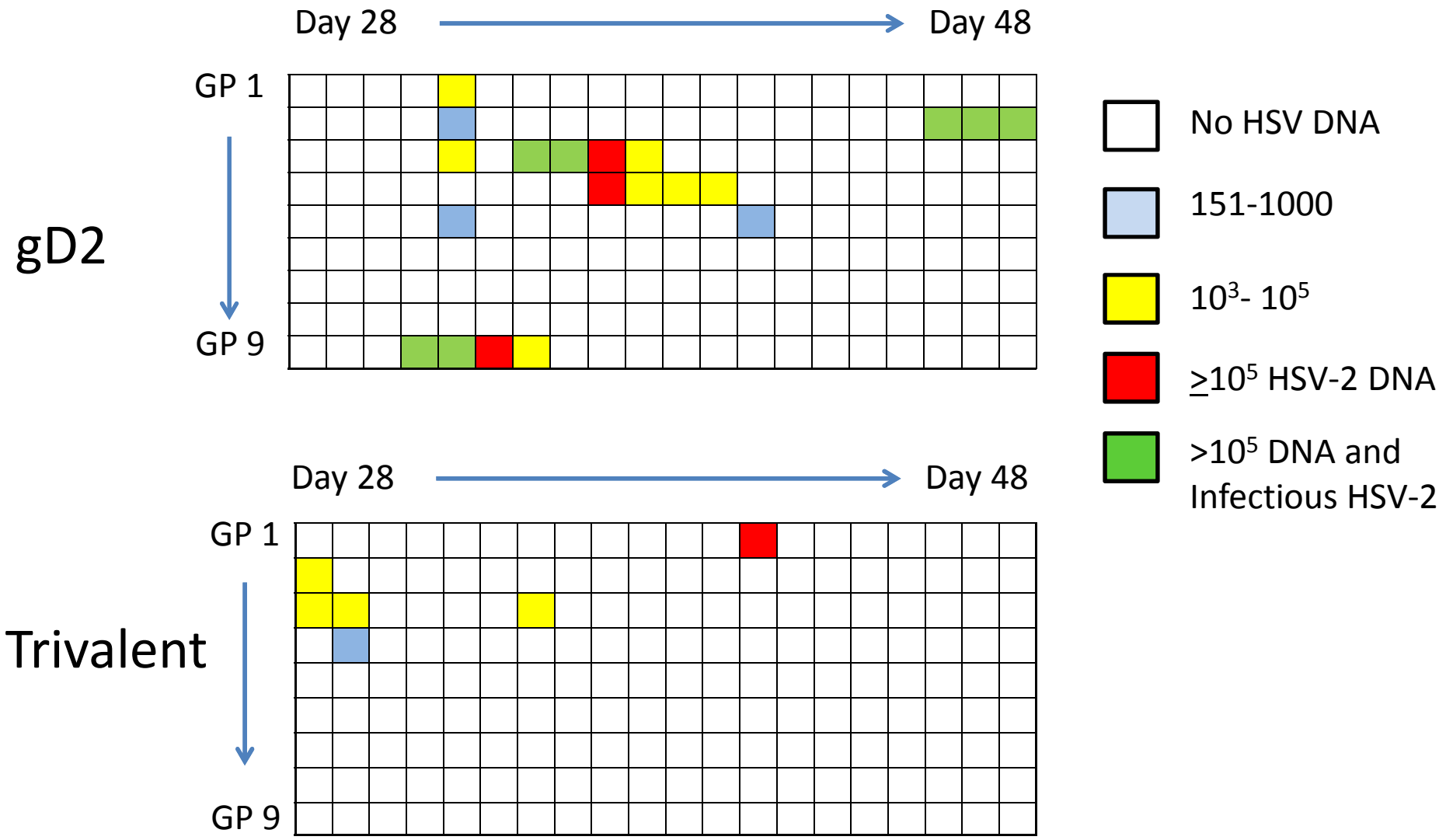
Table. Recurrent disease

	Mock	gD2	gC2/gD2/gE2
Incidence of recurrent genital disease	7/8 (88%)⌘	16/25 (64%)**	10/36 (28%)**
Days with lesions/total observation days (15-60)	85/340 (25%)¶	77/1087 (7%)***	16/1509 (1%)***



Results: Trivalent vaccine highly protective against recurrent disease.

HSV-2 DNA and infectious virus from vaginal swabs





Summary of efficacy studies in guinea pigs

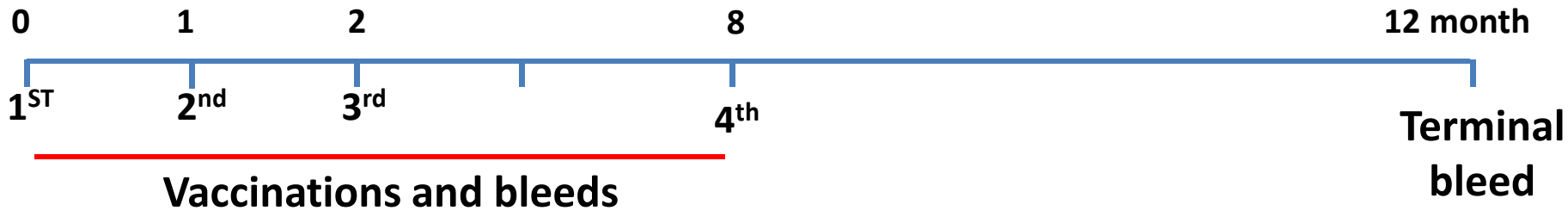
Acute and recurrent disease: Trivalent vaccine is very effective at preventing acute and recurrent genital disease and comes close to meeting **0 acute – 0 recurrent** disease goals.

Asymptomatic DNA shedding: Trivalent vaccine reduced vaginal HSV-2 DNA shedding, but did not eliminate it. However, no infectious HSV-2 was recovered from vaginal swab during recurrent phase in trivalent vaccinated guinea pigs.

Translational efforts: Immunogenicity in rhesus macaques



Collaboration with Tulane National Primate Center



Immunizations and assessments

Groups

Mock (CpG and alum) n=2
gC2 (CpG and alum) n=2
gD2/gC2/gE2 (CpG and alum) n=2

Immunogenicity Endpoints

ELISA antibody titers for each antigens, mucosal antibody titers, neutralization titers, cellular immune responses, C3b blocking ability, Fc receptor blocking ability.

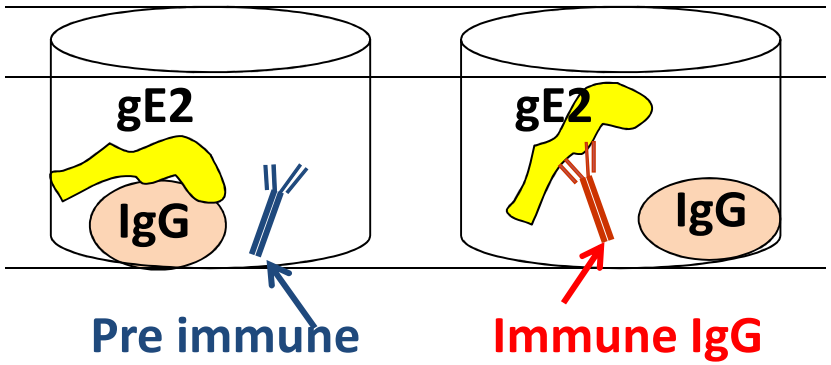
Plasma antibody and neutralization titers in vaccinated rhesus macaques

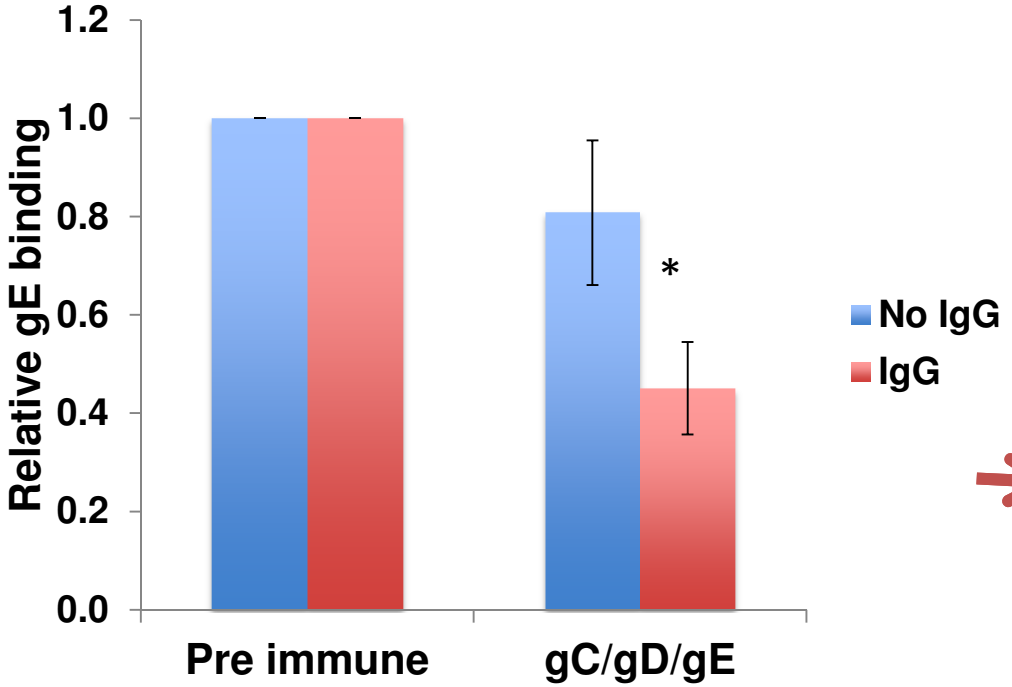
Rhesus #	Treatment	ELISA titers			Neutralization titers	
		gD	gC	gE	3rd imm	4th Imm
1	CpG/alum	<1:50	<1:50	<1:50	<1:20	<1:20
2	CpG/alum	<1:50	<1:50	<1:50	<1:20	<1:20
3	gC2-CpG/alum	NA	1:32000	NA	1:80	NA
4	gC2-CpG/alum	NA	1:32000	NA	1:80	NA
5	Trivalent vaccine	1:32000	1:32000	1:32000	1:80	1:640
6	Trivalent vaccine	1:16000	1:32000	1:32000	1:320	>1:640

ELISA and neutralization titers in vaginal mucosa

Rhesus #	Treatment	Mucosal IgG (ELISA Ab titers)			Mucosal Neutralization titers HSV-2
		gD	gC	gE	
1	CpG/alum	<1:25	<1:25	<1:25	<1:10
2	CpG/alum	<1:25	<1:25	<1:25	<1:10
3	gC2-CpG/alum	NA	ND	NA	ND
4	gC2-CpG/alum	NA	ND	NA	ND
5	Trivalent vaccine	1:400	1:200	1:100	1:10
6	Trivalent vaccine	1:6400	1:1600	1:400	1:80

Blocking of gE binding to the Fc end of human IgG by vaccinated rhesus IgG

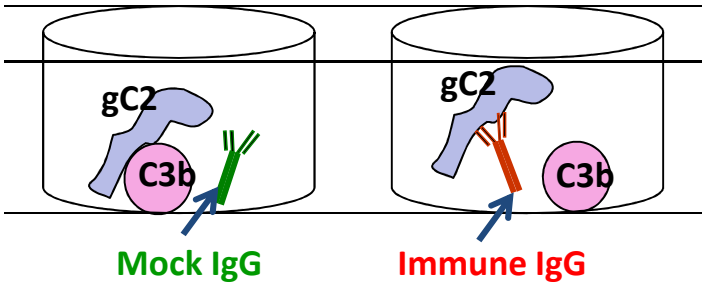


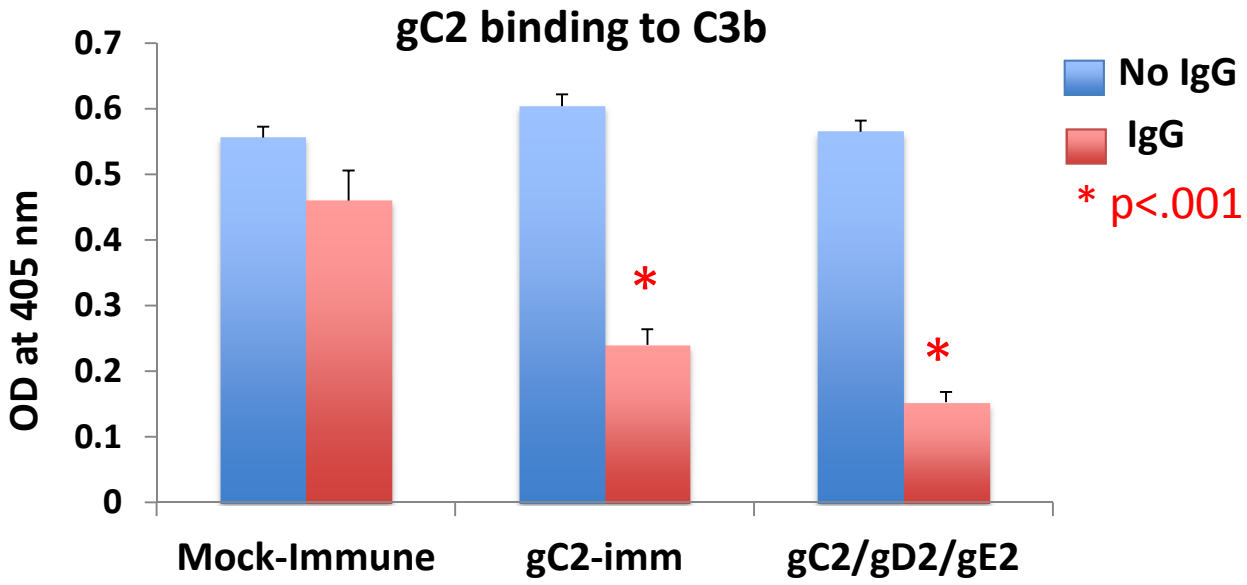


- Plate was coated with human IgG from an HSV-1/2 negative donor
- gE2 was incubated with rhesus IgG (0 or 200ng/μl) at 37°C for 1h then added to IgG-coated wells
- Bound gE was detected with polyclonal rabbit anti-gE2 Ab
- * P<0.001

→ **Blocking of gE2 binding to IgG Fc will enhance neutralizing ability of gD2 antibody**

Blocking of gC2 binding to C3b by vaccinated rhesus IgG

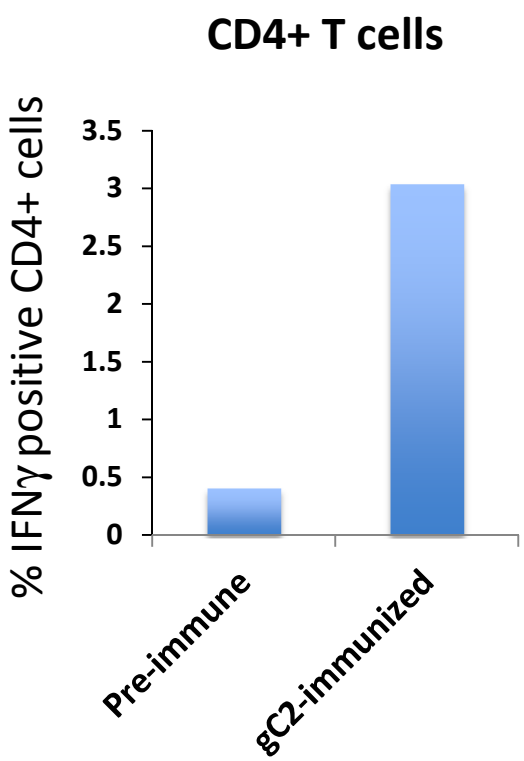


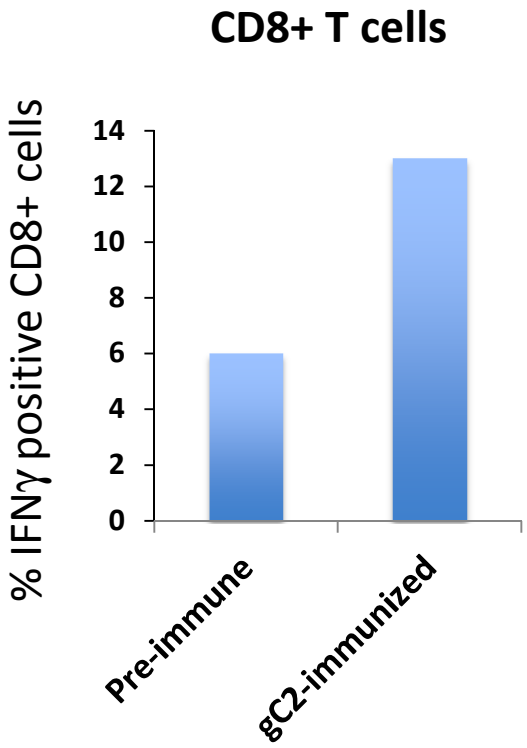


- Plate was coated with C3b
- gC2 was incubated with rhesus IgG (3rd bleed) at 37°C for 1 h then added to C3b-coated wells
- Bound gC was detected with polyclonal rabbit anti-gC2 Ab

→ **Antibodies that block gC2 binding to C3b will enhance neutralizing ability of gD2 antibody**

Cellular immune response in gC2-immunized Rhesus macaques

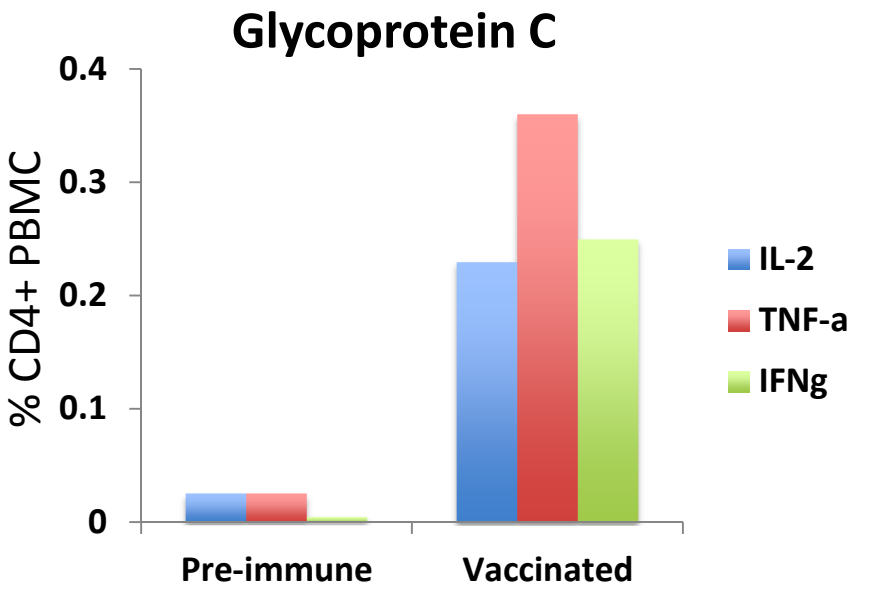


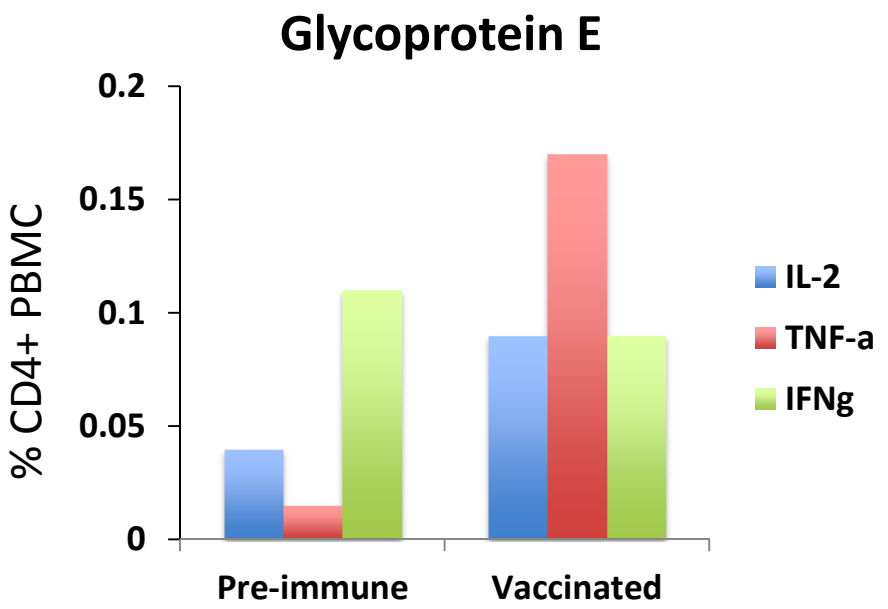


- PBMCs were isolated following pre and post-immunizations.
- Cells were stimulated with a pool of overlapping peptides (gC2).
- IFNγ positive CD4 and CD8 cell were measured by surface and intracellular staining, followed by FACS analysis

gC2 immunized rhesus PBMC had increased IFNγ positive CD4 and CD8 T cells when stimulated with gC2 peptide pool.

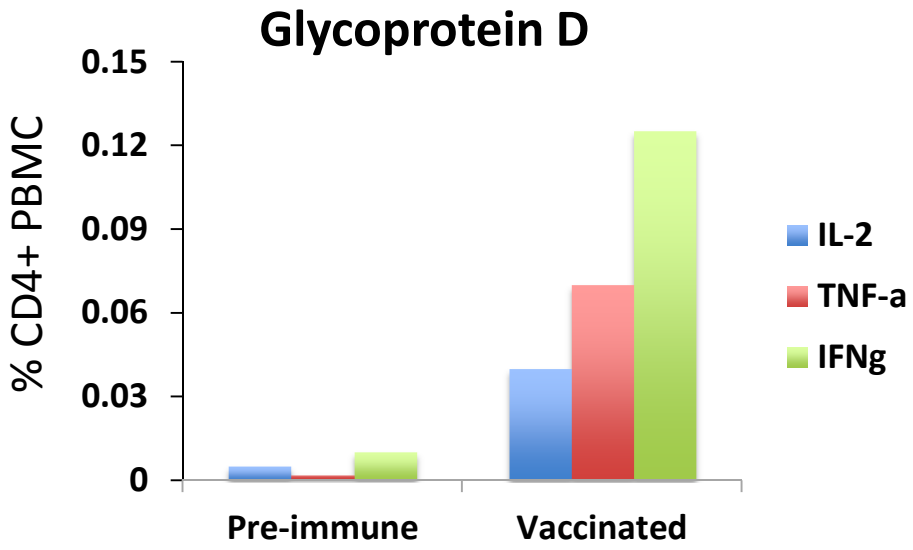
Vaccine specific CD4+ T cell responses in rhesus





- PBMCs were isolated following pre and post-immunizations.
- Cells were stimulated with purified gC2, gD2 or gE2.
- IL-2, TNF-α, and IFNγ, positive CD4+ cell were measured by surface and intracellular staining, followed by FACS analysis

→ **Immunogen specific CD4+ T cell responses are induced in vaccinated rhesus**



Summary

- Trivalent vaccine is highly effective in eliminating primary and recurrent disease in guinea pigs.
- High level ELISA titers to gD, gC and gE are detected
- High level of neutralizing antibody titers are noted in plasma followed by trivalent vaccine immunization
- Vaccine specific mucosal IgG are detected in rhesus vagina
- C3b blocking and FcR blocking antibodies are produced in vaccinated rhesus
- CD4 and CD8 T cell responses are induced in vaccinated rhesus

Conclusions

→ A trivalent vaccine that blocks evasion of host immunity is a promising candidate from future human trials.

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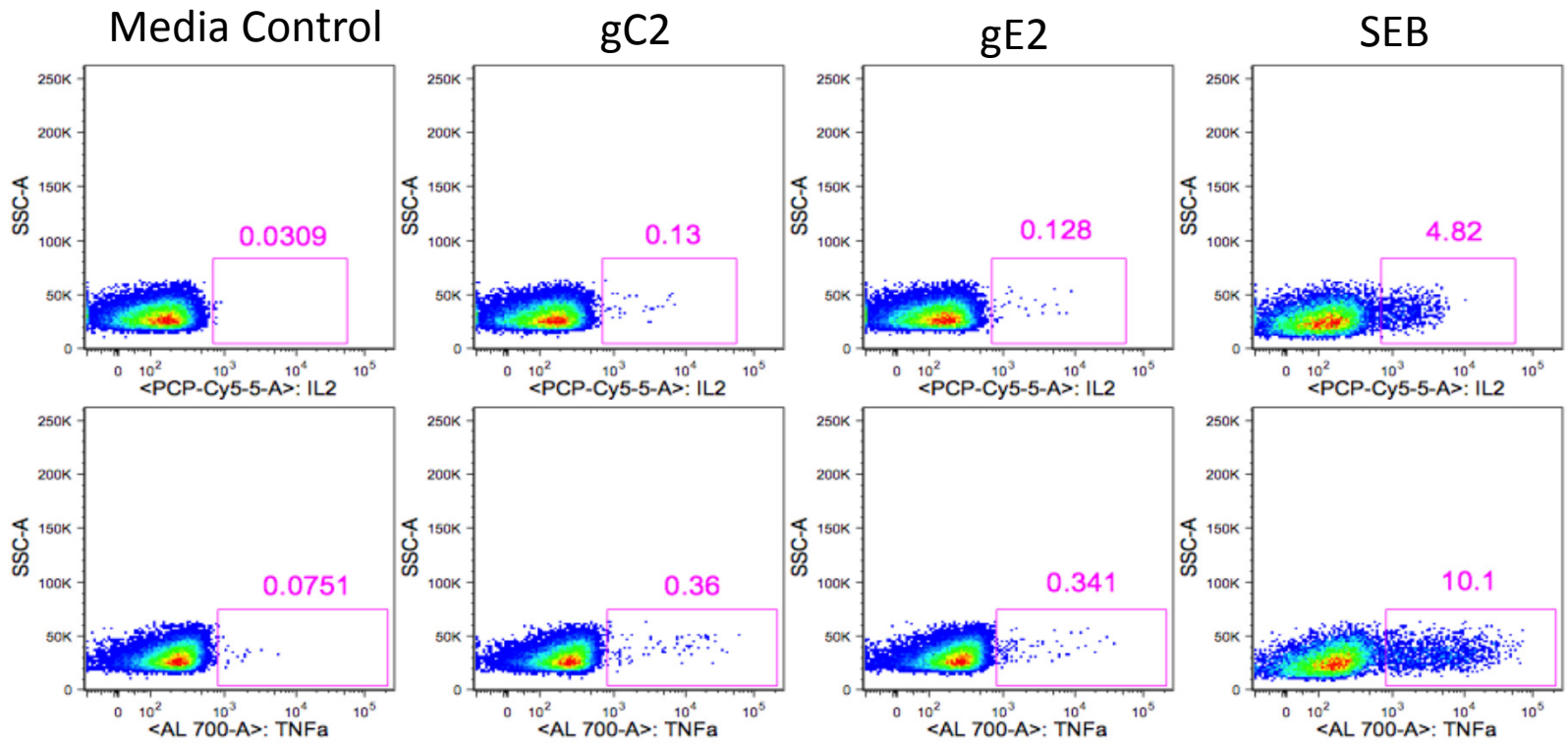
GSK

Funding: NIH, Merck (2007-09), CFAR





CD4+ T cell responses



Animal IH34
Sample date
4/22/2014