

**Invaplex: A Safe Nasal Adjuvant for
Enhancing Mucosal and Systemic
Antibody Responses to
DNA/protein Vaccines**

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Improving efficacy of HIV “CTL-inducing” DNA vaccines

Strategies:

- Formulate with envelope protein to induce antibodies.
- Administer by the nasal route to generate genital tract & intestinal immune responses.

Challenge:

- Protein immunogen will require adjuvant.
- No safe nasal adjuvants for humans.

Invaplex: a nasal *Shigella* vaccine

Developed by Dr. Edwin Oaks (Walter Reed)

Macromolecular complex:
Shigella flexneri
lipopolysaccharide +
IpaB & IpaC invasins.



- Invaplex traffics within the host cell from early and late endosomes to the Golgi apparatus, with eventual release into the host cell cytosol.
- Uptake is non-toxic to host cell.

Invaplex: a nasal *Shigella* vaccine & safe nasal adjuvant?

- Highly safe in human nasal cavity:
 - Phase I gradual escalation dose study: No adverse side effects in doses up to 690 µg.
- Functions as a nasal adjuvant for proteins in mice (*Infect Imm* 2006;74:2856).

Objective

Test Invaplex for nasal adjuvant activity in nonhuman primates using a nasal DNA/protein vaccine formulation.

DNA vaccine & protein immunogen

pVacc4 SHIV DNA (Anna Aldovini):

- HIV89.6 env, SIV_{mac239} gag, pol.
- noninfectious, structurally intact viral particles.
- MVA-SHIV nasal boosting of A*01+ Mm nasally-primed with SHIV DNA elicits gag-specific CD8 T cells in blood & rectum but low antibody (*Jl 2004; 172:3745*).

SIV_{mac251} rgp130 protein (ImmunoDiagnostics):

- monomeric.
- glycosylated (baculovirus-derived).

Protocol for testing Invaplex nasal adjuvanticity

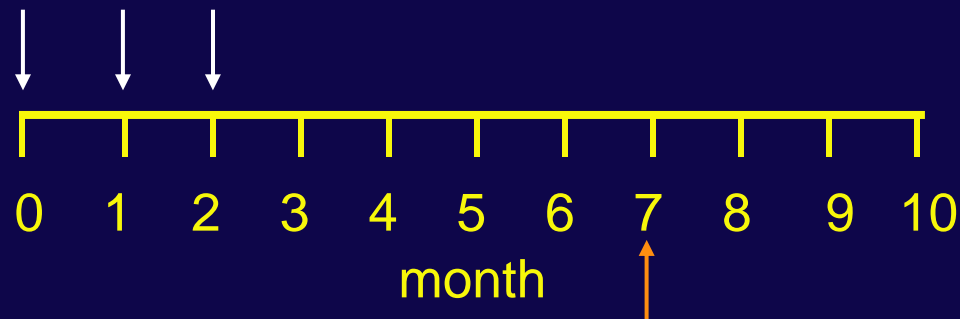
Animals: 12 female rhesus macaques (A*01-)

Vaccinations 1-3: nasal, monthly intervals

Gp 1: 1.5mg SHIV DNA + 0.5mg gp130

Gp 2: 1.5mg SHIV DNA + 0.5mg gp130 + 0.25mg Invaplex

Gp 3: 1.5mg SHIV DNA + 0.5mg gp130 + 0.50mg Invaplex



Nasal Boost:

2×10^{10} PU rAd5-SIV env
+
 2×10^{10} PU rAd5-SIV gag
(replication-incompetent)

NIH Vaccine
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Immune Assessments

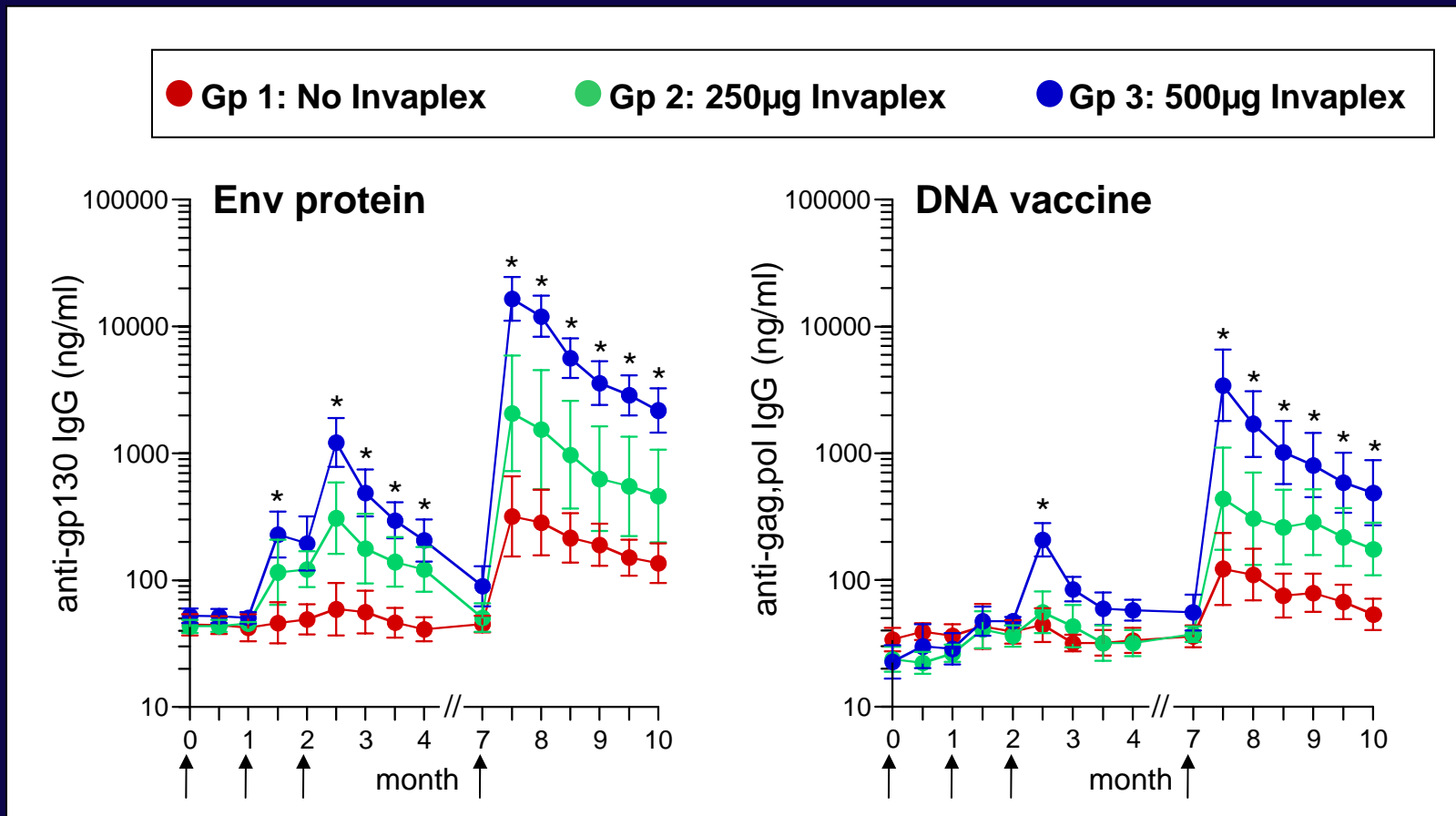
ELISA for antibodies:

- SIV rgp130 (protein immunogen)
- SIV “gag,pol” lysate (DNA encoded antigens)
 - IgA antibodies in secretions (nasal, rectal, cervicovaginal).
 - IgG and IgA antibodies in serum.

Intracellular cytokine staining for T cells in blood:

- overlapping SIV env peptides (for protein)
- overlapping SIV gag peptides (for DNA)
 - IFN- γ , IL-2, TNF- α , IL-4
 - CD4 and CD8 T cells

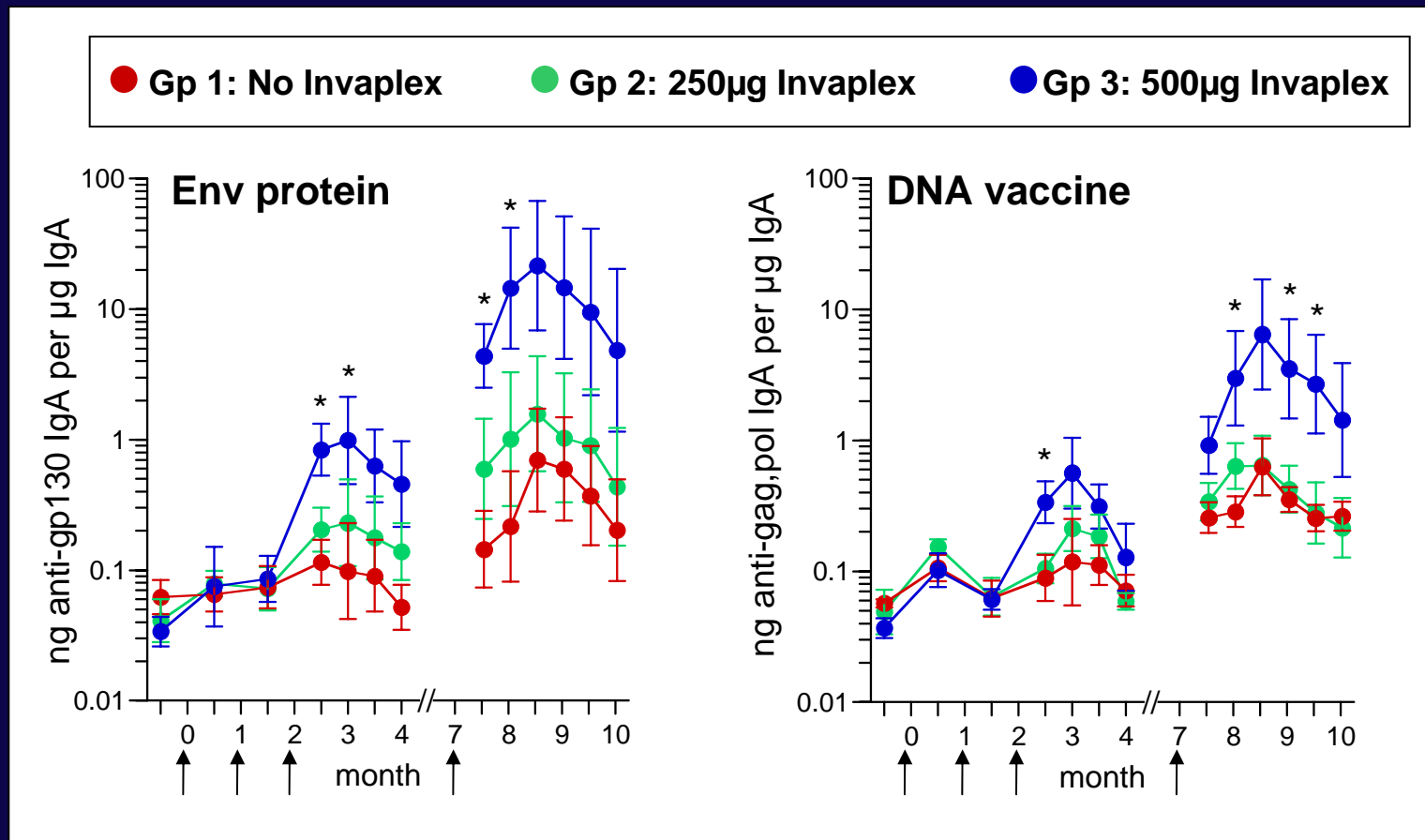
IgG antibodies in serum



Shown: geometric mean \times/\div SEM.

***Gp3 significantly > Gp 1 by Mann-Whitney**

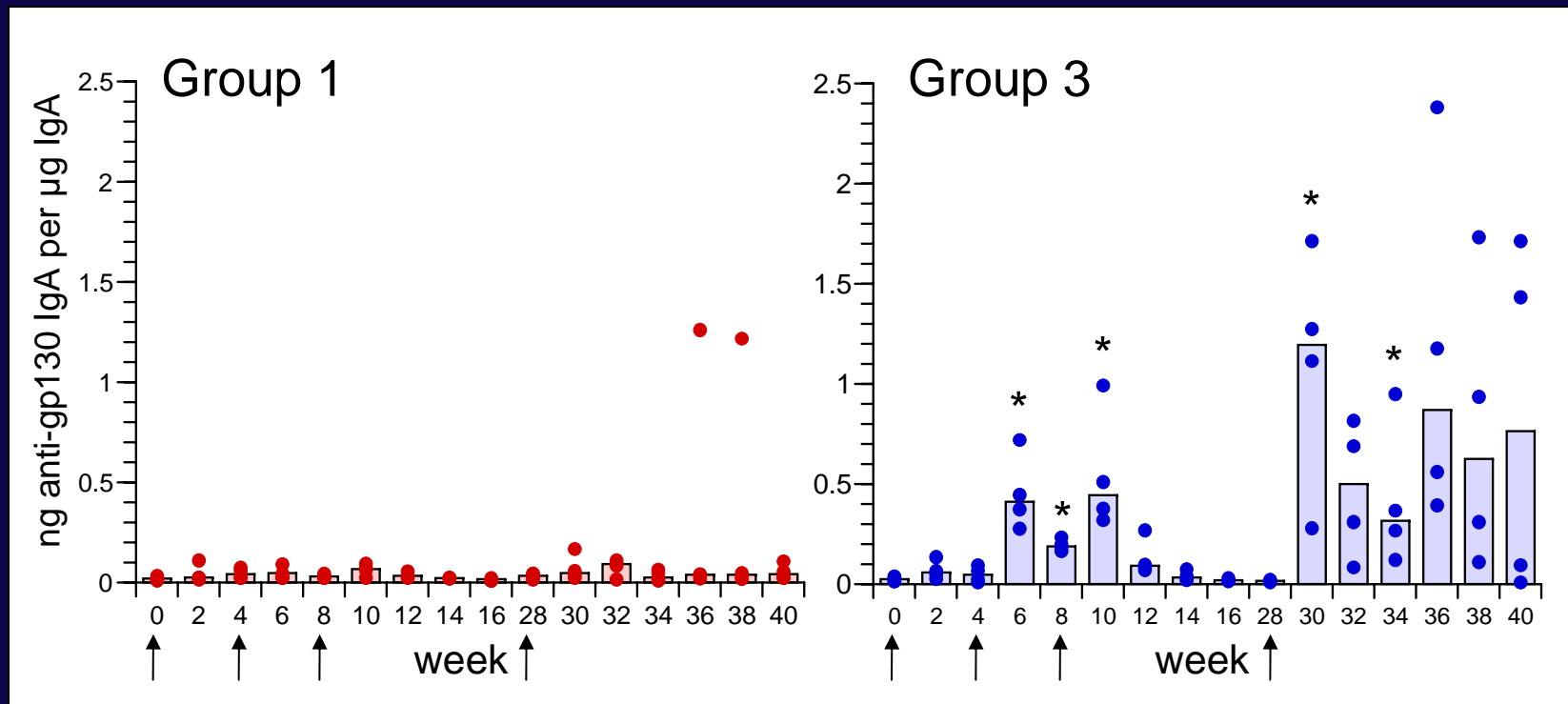
IgA antibodies in nasal secretions



Shown: geometric mean \times/\div SEM.

***Gp3 significantly > Gp 1 by Mann-Whitney**

Invaplex enhances levels of gp130-specific IgA in vaginal secretions



Columns represent medians.

Gp2 responses were intermediate.

**Gp3 significantly > Gp1 by Mann-Whitney.*

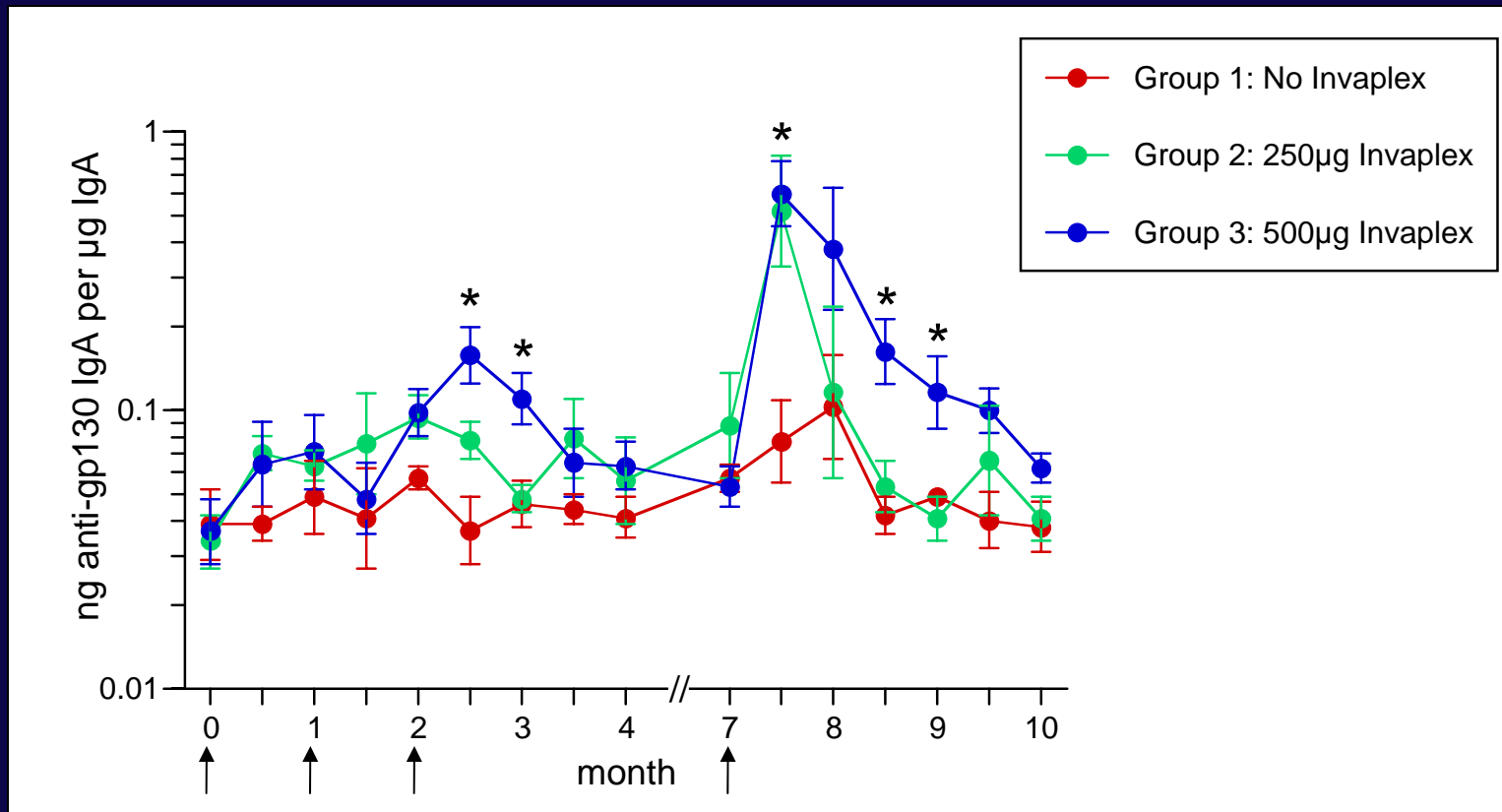
Increased frequency of gag,pol-specific IgA antibodies in Gp3 vaginal secretions

Group & Mm		ng anti-gag,pol IgA per µg total igA													
		wk0	wk4	wk6	wk8	wk10	wk12	wk14	wk16	wk28	wk30	wk32	wk34	wk36	wk38
73-76	73	0.016	0.050	0.142	0.044	0.081	0.038	0.041	0.030	0.015	0.013	0.013	0.004	0.015	0.016
	74	0.017	0.118	0.173	0.137	0.624	0.128	0.138	0.044	0.027	0.039	0.079	0.032	0.044	0.065
	75	0.031	0.096	0.110	0.064	0.383	0.082	0.044	0.035	0.026	0.142	0.101	0.077	0.129	0.109
	76	0.031	0.032	0.076	0.089	0.100	0.052	0.043	0.026	0.007	0.105	0.040	0.035	0.016	0.022
77-80	77	0.024	0.072	0.084	0.120	0.063	0.053	0.019	0.042	0.009	0.114	0.014	0.100	0.021	0.033
	78	0.049	0.032	0.088	0.107	0.044	0.024	0.024	0.058	0.081	0.475	0.123	0.026	0.031	0.100
	79	0.017	0.051	0.058	0.110	0.045	0.048	0.014	0.033	0.075	0.050	0.087	0.033	0.012	0.044
	80	0.030	0.012	0.041	0.043	0.042	0.044	0.009	0.032	0.015	1.837	0.338	0.358	0.098	0.032
81-84	81	0.027	0.262	0.136	0.617	1.349	0.033	0.046	0.022	0.013	0.684	0.165	0.146	0.108	0.030
	82	0.010	0.026	0.191	0.055	0.244	0.075	0.089	0.057	0.033	0.198	0.844	1.279	0.335	0.181
	83	0.060	0.069	0.095	0.061	0.112	0.042	0.021	0.062	0.038	3.702	1.435	0.797	0.441	0.115
	84	0.014	0.101	0.423	0.958	0.358	0.437	0.270	0.106	0.002	0.481	0.265	0.183	0.085	0.091

High-lighted, boxed values represent rectal secretions containing significant specific activity.

To be significant, specific activity had to be ≥ 0.158 which represents the mean specific activity (0.044) + 3 SD determined for rectal secretions of naive Mm.

Invaplex enhances levels of gp130-specific IgA in rectal secretions

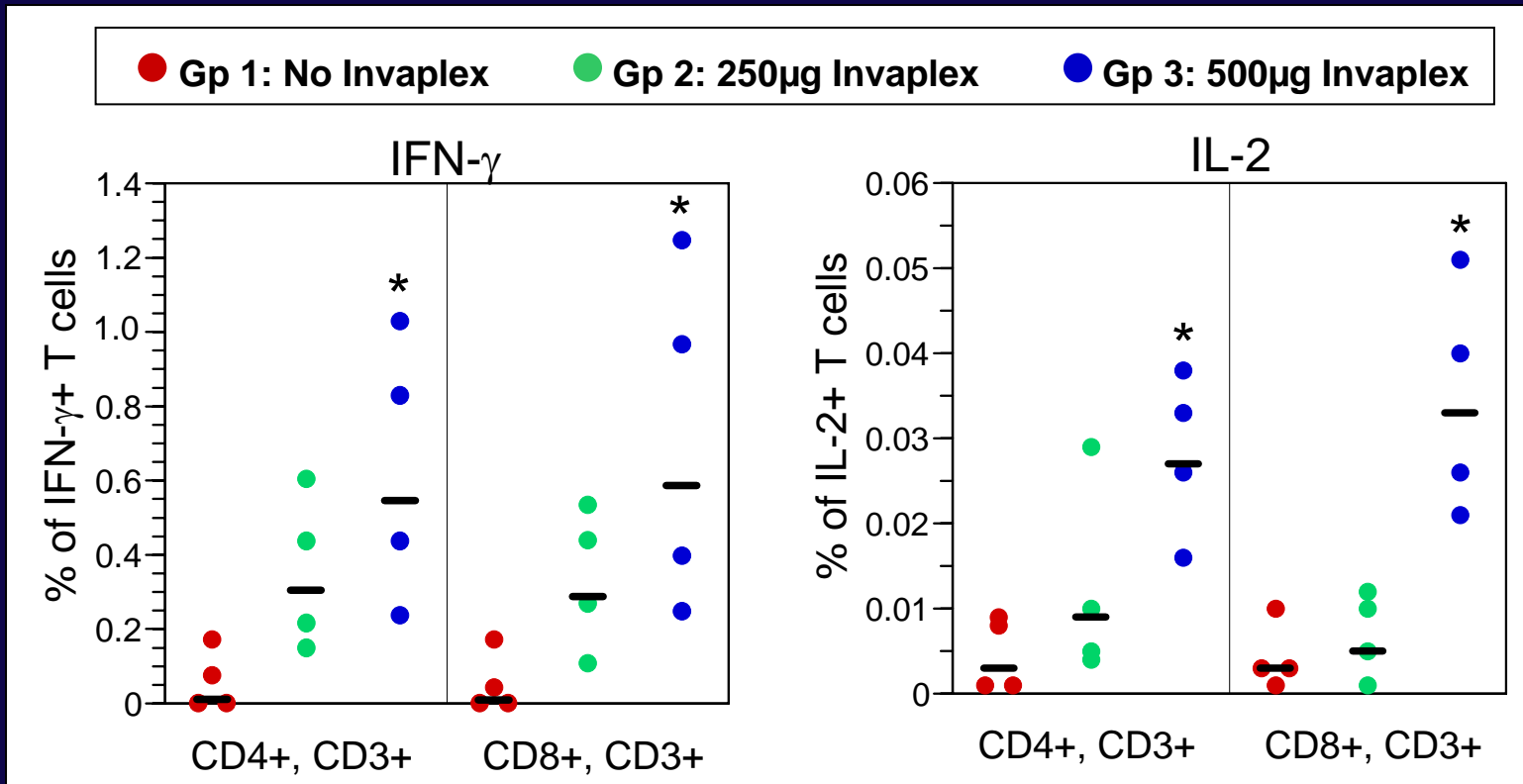


Shown: geometric mean \times/\div SEM.

***Gp3 significantly > Gp 1 by Mann-Whitney.**

Invaplex increases percentages of SIV env-specific T cells in blood

1 mo after DNA/protein imm #3



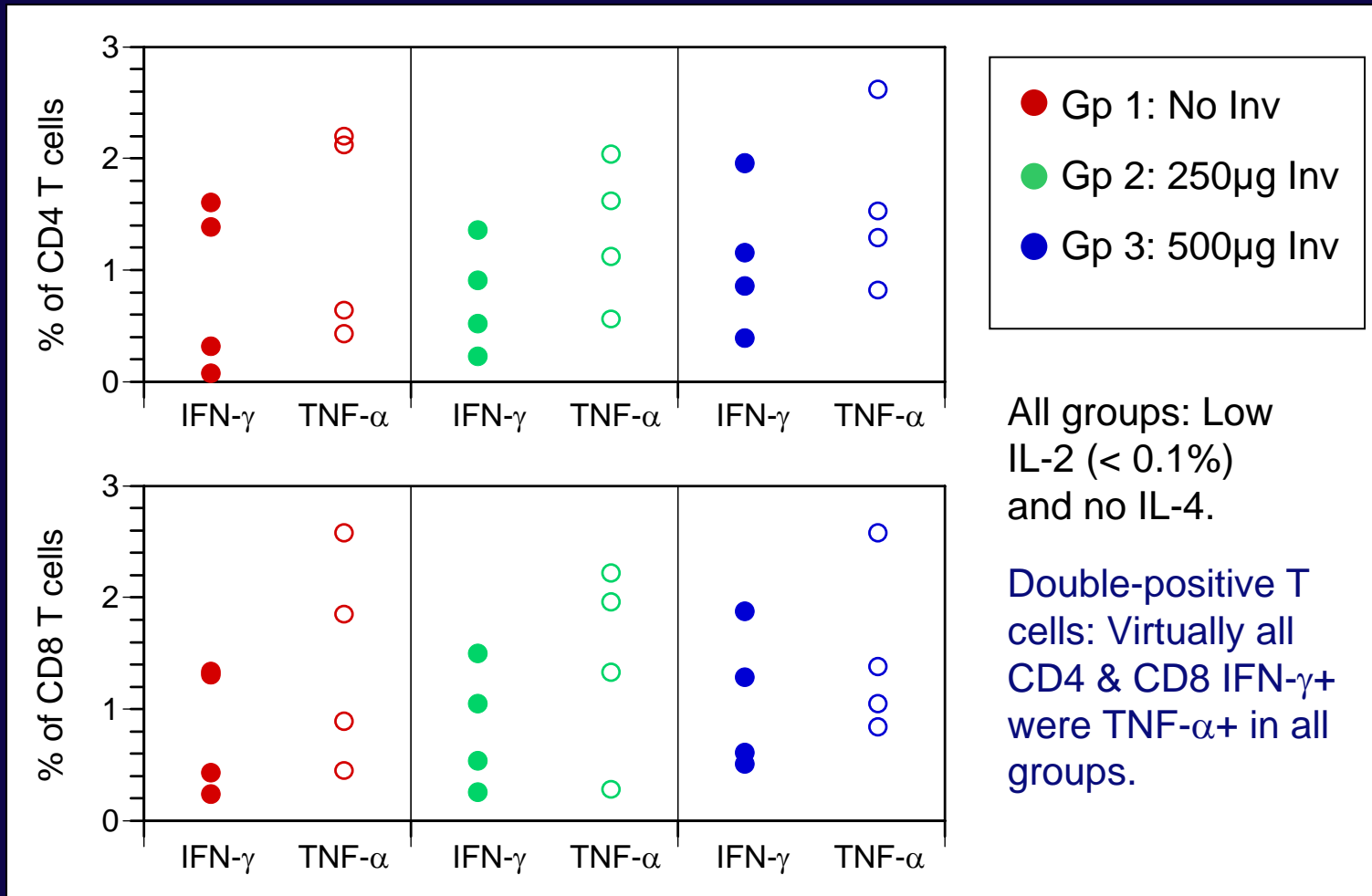
Bars represent geometric means.

No detectable TNF- α or IL-4.

*Gp3 significantly > Gp 1 by Mann-Whitney.

SIV gag-specific T cells in blood

2 mo after boosting with Ad-SIV gag



Conclusions

- Invaplex does function as a nasal adjuvant for co-administered protein antigen in nonhuman primates.
 - increases levels of protein-specific IgG in serum.
 - enhances levels of protein-specific IgA in local and distal mucosal secretions.
 - increases frequency of protein-specific CD4 and CD8 T cells secreting Th1-type cytokines in blood.
- **Invaplex can also adjuvant systemic and mucosal antibody responses to nasal DNA vaccines.**

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