Rapporteur Session
AIDS Vaccine 2008

B cell immunology, Neutralizing antibodies, Mucosal immunology: a summary

Ralph Pantophlet, PhD
Faculty of Health Sciences
Simon Fraser University

SFU SIMON FRASER UNIVERSITY
THINKING OF THE WORLD

Selected highlights

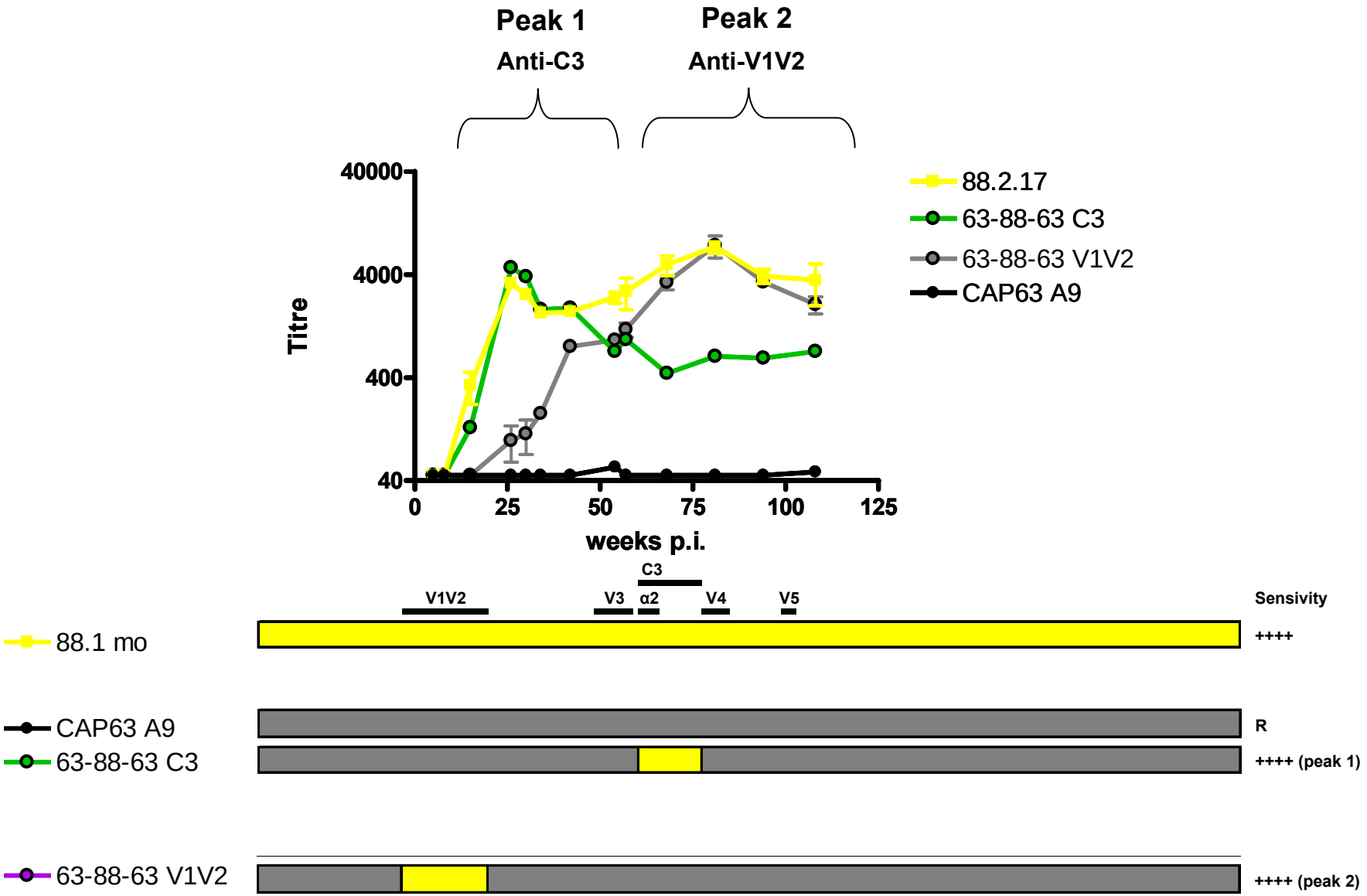
- Analysis/dissection of nAb responses in infected individuals and immunized animals
- Anti-lipid antibodies ~ MPER Abs
- Mucosal immunization strategies
- Strategies to isolate and characterize humoral responses at the B cell level

Analysis and dissection of nAb responses in infected individuals and immunized animals

Moore et al. (OA03-04)

- Neutralization escape patterns in 2 subtype C-infected individuals suggest that low number of (homologous) nAb specificities develop during course of infection
- Individual#1: escape in V5 (and V1/V2)
- Individual#2: escape in C3 and V1/V2
- NAb specificities appear to develop sequentially (shift of nAb response to new epitopes)
- Accumulation of mutations at certain sites suggests that HIV possibly has multiple pathways for nAb escape
- Targeting multiple epitopes with a vaccine required

Multiple nAb specificities



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- **Binley et al. (S01-01); Li et al. (P04-37):** Use of various assays to dissect nAb responses (e.g. 'modified' neut assay, virus capture assay, neut inhibition assay, BN-PAGE, serum fractionation)

  => applied to Hum and Rab sera – unknown nAb specificities apparent (20-40%); generation of more mutant proteins may be needed

- **Corti et al. (S01-05); Simek et al. (P04-22):** Use of novel EBV transformation techniques to isolate novel nAbs from non-B infected individuals

  => 47 antibodies so far, a few promising (e.g. HK20 to gp41); algorithms developed to rapidly identify individuals with potent/broad nAb activity

- **Warren et al. (SP02-02):** 'In vitro' immune system to screen Ags for their potential to elicit x-nAb responses

  => analyses ongoing to establish system; class-switching of Abs current limitation

- **Bunnik et al. (P04-05); Gils et al. (P04-06):** Analysis of autologous nAb from SC to AIDS symptoms

  => early escape coincides with increased V-loop length; as nAb pressure wanes, virus often reverts wt Env sequence (shorter V-loop length and fewer # of PNGS); no difference between LTNPs and progressors in development of x-nAb responses

- **Shen et al. (P04-32); Gray et al. (LB-19):** Identification of select individuals with anti-MPER nAb activity

  => Use of peptide absorption to isolate MPER-specific serum fraction; 2F5- (Shen et al.) and 2F5/4E10-like (Gray et al.) activity observed. Gray et al. show using neut inhibition assay that anti-MPER Abs not responsible for neut against all viruses (3/9 viruses neutralized by other specificities)

- **Scarlatti et al. (P04-09):** Comparison of in vitro neutralization assays

  => Difficulties due to cell donor-to-donor variation and virus source

Anti-lipid antibodies ~ MPER Abs

Moody et al. (OA03-06)

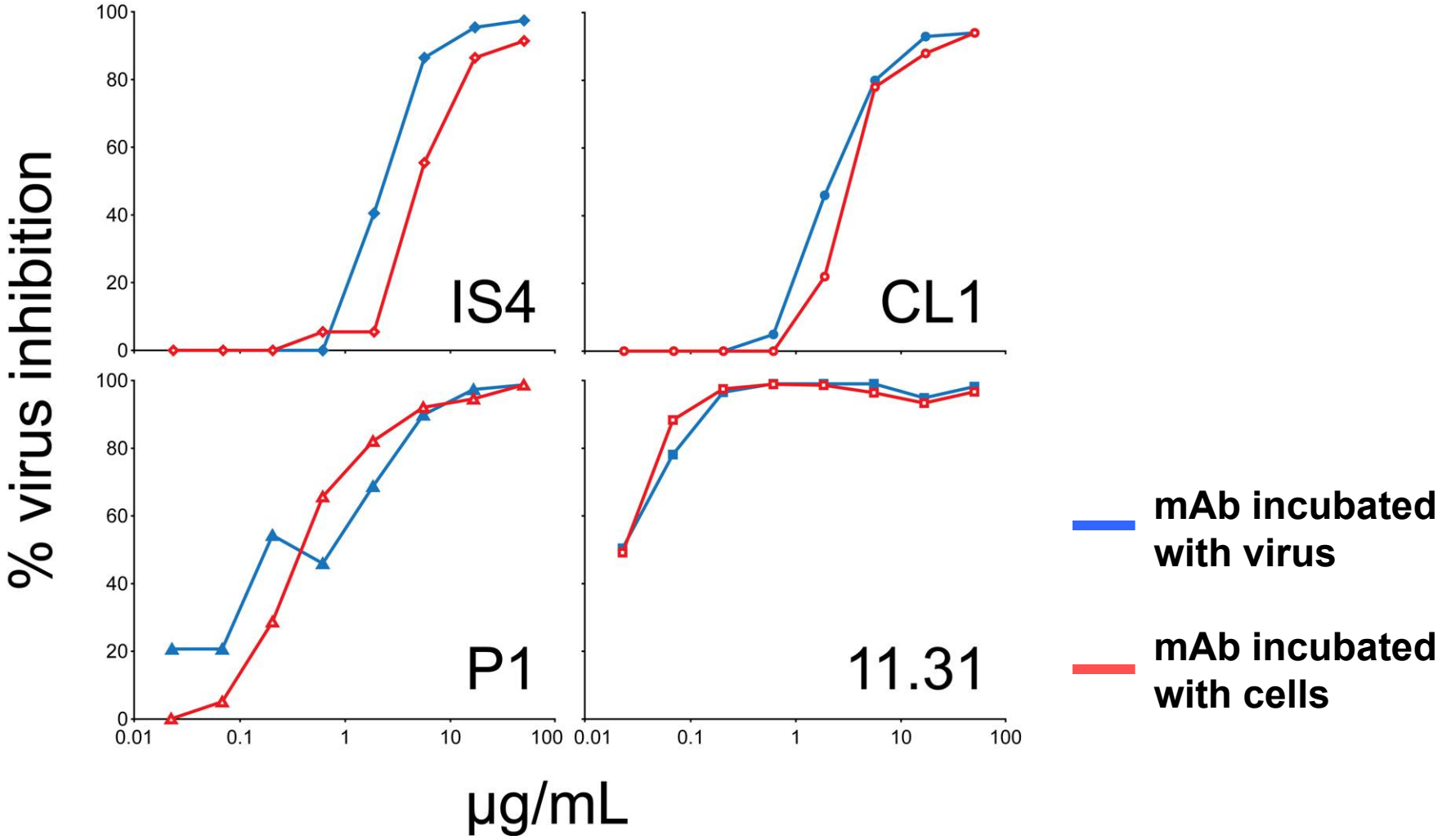
- AIDS is rare in individuals with autoimmune disease – role of anti-lipid Abs? (vis-à-vis lipid binding by MPER Abs)
- Anti-lipid Abs isolated from autoimmune disease patients; two groupings based on β2-gp1 dependence for lipid binding
- Neut observed in PBMC assay, not TZM-bl; only Abs that bind independent of β2-gp1
- Inhibition of infection due to interaction with target cells alone
- Further analysis revealed that lipid interaction induces chemokine production (MIP1α, MIP1β), resulting in HIV infection blockage – blockage can be revered using anti-chemokine Abs
- Challenge studies in primates planned
- Induction of anti-lipid Abs (β2-gp1-independent) a possible stragety for vaccine design?

Anti-Lipid Antibodies Inhibit HIV-1 Primary Isolates With Greater Breadth than 2F5, 2G12 and b12

	IC80 values in µg/mL					
HIV-1 isolates	**IS4**	**CL1**	**P1**	**11.31**	**Anti-RSV**	**Tri-Mab**
B.TORNO	**0.6**	**0.7**	**17**	**0.09**	>50	**0.03**
B.PVO	**0.4**	**0.2**	**4.5**	**0.03**	>50	**0.64**
B.6535	**0.07**	**0.4**	**4.0**	**0.14**	>50	>25
C.DU123	**0.06**	**0.2**	**1.7**	**<0.02**	>50	>25
C.DU156	**2.8**	**2.6**	**16**	**0.06**	>50	>25
C.DU151	**1.8**	**4.1**	**0.1**	**<0.02**	>50	>25
C.DU172	**1.1**	**0.6**	**0.55**	**<0.02**	>50	>25
SHIV 162P3	**5.2**	**1.2**	**1.6**	**0.06**	>50	**1.5**

Tri-Mab = 2F5, 2G12, IgG1b12

Anti-Lipid Antibodies Inhibit HIV-1 Infectivity By Binding to Uninfected Target PBMC



David Montefiori, Duke

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- **Haynes/Alam et al. (OA07-06); Scherer et al. (P05-04):** Role of hydrophobic CDR H3 of MPER Abs in lipid interaction

  => mutants generated in which specific hydrophobic residues in CDR H3 are changed; select changes reduce gp41 (peptide) binding; some changes result in loss of lipid interaction and loss of neut activity against HXB and primary isolates *Haynes/Alam propose 2-step mode of MPER interaction

- **Cho et al. (OA07-05); Ofek et al. (OA07-07):** Structure-based design of antigens to elicit MPER Abs, e.g. 2F5 (Ofek)

  => computational design heavily used to produce thermo-stable structures that resemble Ab-bound state; *however, so far none of the strategies have incorporated lipid context*

- **Holl et al. (AO07-08):** Rarity of neutralizing MPER Abs investigated using RAG1-/- mice reconstituted with CD B-cells

  => results suggest possible tolerance mechanisms in bone marrow that prevent development of B cells with (low) affinity for MPER antigens; *however, how specific tolerance of B cells occurs that may produce x-neut MPER Abs unclear*

- **Clark et al. (SP02-01):** Vector-mediated Ab gene delivery to control or prevent HIV infection (using rAAV system)

  => proof-of-concept completed; 6/9 animals protected with model Ab (scFv-IgG2 construct); non-protected animals develop anti-Ab response; future plans to use HIV bNAbs; *may allow for better study of anti-lipid Ab application*

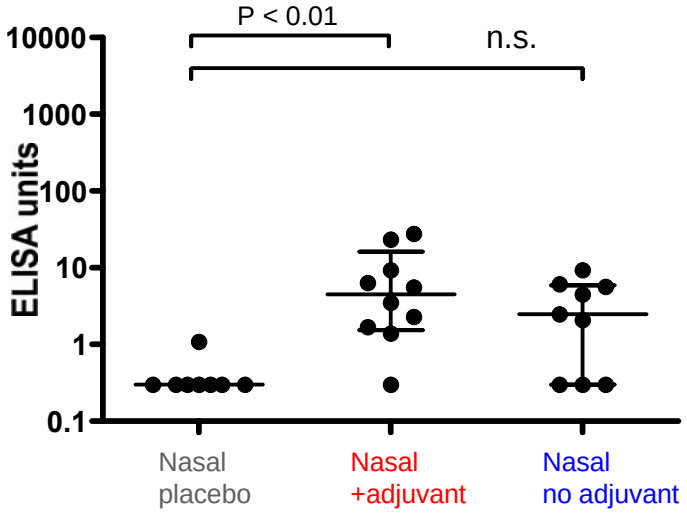
Mucosal immunization strategies

Lewis et al. (OA04-04)

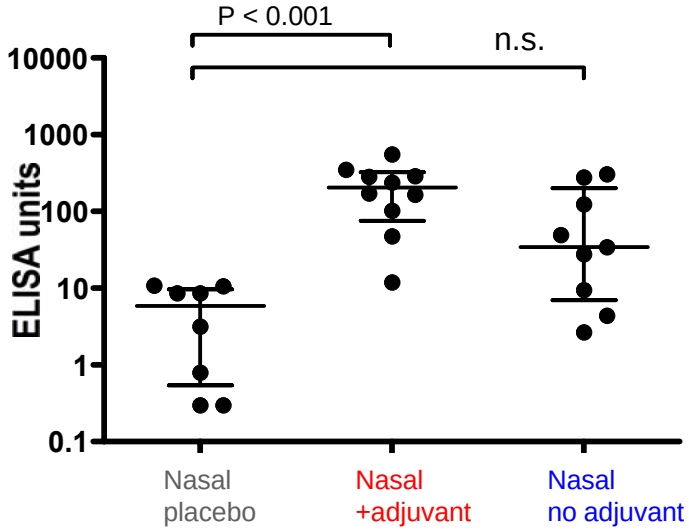
- Nasal immunization in humans (nasal prime/i.m boost) with o-gp140ΔV2 (SF162) adjuvanted with cholera toxin/LT toxin (Novartis LTK63; nasal) and MF59 (i.m.)
- Immunization strategy: 3x i.n., 2x i.m.
- High levels of systemic IgG; relatively lower levels of cervical and vaginal IgG observed; also IgA
- Use of LTK63 for nasal delivery not ideal; other routes perhaps better
- Only homologous neutralization tested

Effect of nasal priming with or without LTK63 adjuvant on serum anti-gp140V2LD IgG response to IM boosters

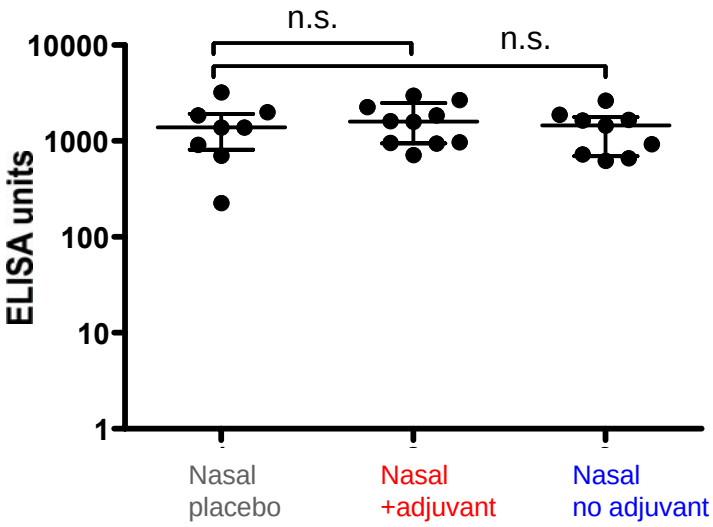
After three nasal primes



After first IM boost



After second IM boost



Group 1:
nasal placebo

Group 2:
nasal gp140+LTK63

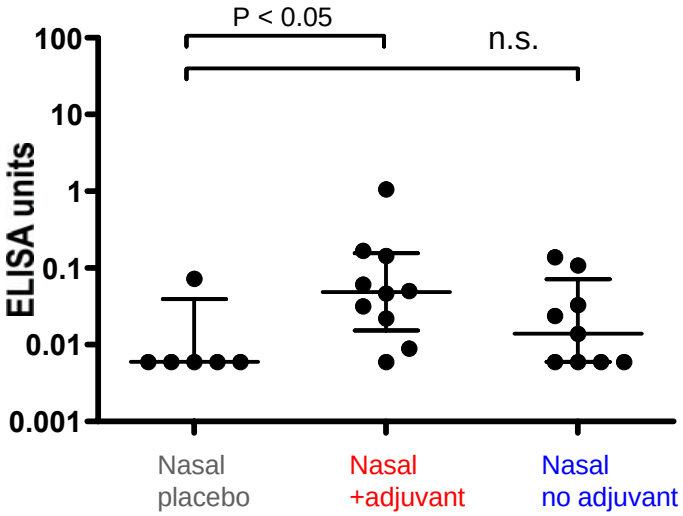
Group 3:
nasal gp140

Medians ± IQR
Kruskal-Wallis statistic
Dunn's Multiple Comparison Test

- ✧Higher response to nasal immunisation and first IM boost after nasal prime with gp140+LTK63
- ✧Trend to more responders with unadjuvanted gp140 nasal prime
- ✧Similar serum IgG response after second IM boost in all groups: MF59 is a potent systemic adjuvant

Effect of nasal priming with or without LTK63 adjuvant on vaginal anti-gp140V2LD IgG response to IM boosters

After three nasal primes



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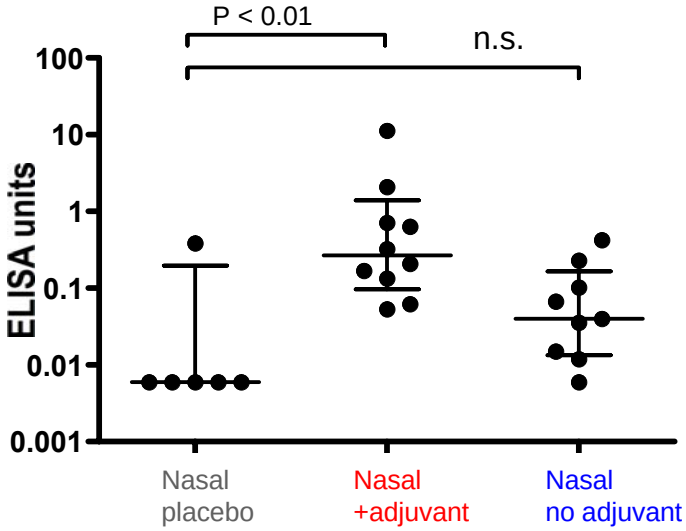
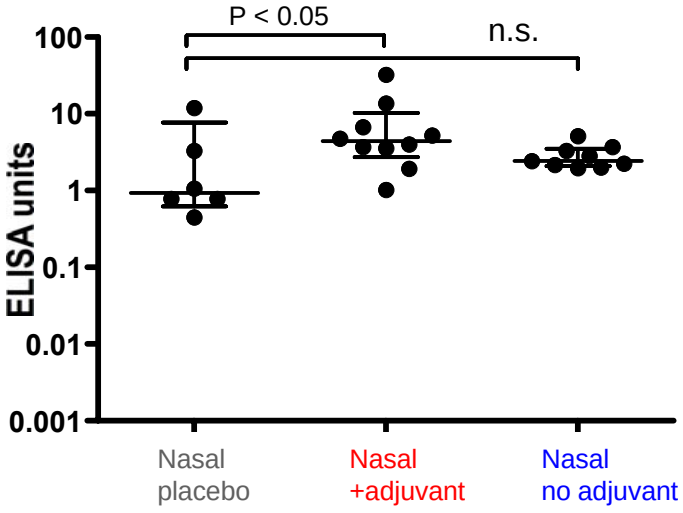


After second IM boost



Group 1:
nasal placebo

Group 2:
nasal gp140+LTK63

Group 3:
nasal gp140

Medians ± IQR
Kruskal-Wallis statistic
Dunn's Multiple Comparison Test

- ✧Higher / more frequent response to nasal immunisation and both IM boosts after nasal prime with gp140+LTK63
- ✧Trend to higher response to IM boost after nasal prime with g140 alone
- ✧IM prime & boost alone induces vaginal IgG responses

- **Kozlowski et al. (OA04-03):** Intranasal adjuvant Invaplex (NHP application)
  => adjuvant increases anti-gp120 IgG levels in serum, IgA in nasal passage, and rectal IgA; adjuvant also increases % of Env-specific T cells; *however, no challenge study performed to study Ab functionality*

- **Mann et al. (P02-02):** Mucosal delivery of antigen using CD71 (Hum Fe2+ scavenger receptor)
  => genetic constructs and chemical conjugation applied (biotin/avidin); vaginal application generates systemic and mucosal (IgG) responses (no response with Ag alone)

- **Fraser et al. (P04-21):** Dose comparison and epitope mapping after mucosal immunization
  => Animals (rabbits) immunized 27 times (divided in 3 cycles over 3 wk period); only IgG measurable in mucosa (no IgA); systemic IgA and IgG; V3 immunodominant

- **Arias et al. (P11-17):** Use of wax nanoparticles as delivery vehicles for Ag for mucosal immunization
  => Ag (TT, gp140) absorbed onto particles ingested by DCs, resulting in induction of mucosal and systemic IgG; adjuvant studies planned

Strategies to isolate and characterize humoral responses at the B cell level

- **Connors et al. (S01-03); Wyatt et al. (S01-06):** B cell clones sorted individually then RT-PCR cDNA (using bio-gp140)

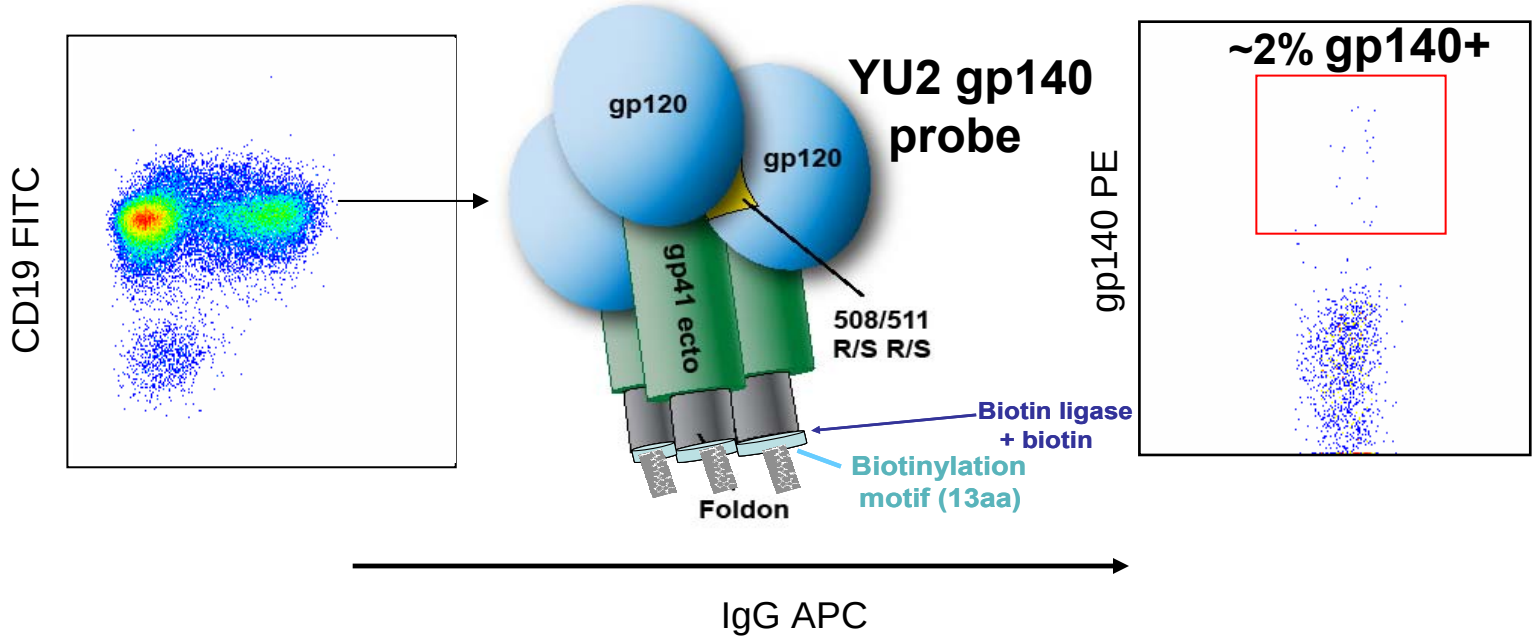
  => results show 80% of cells categorized into clonal families; 500 Abs isolated to date, 160 unique; *none of the Abs isolated so far are highly cross-neutralizing*

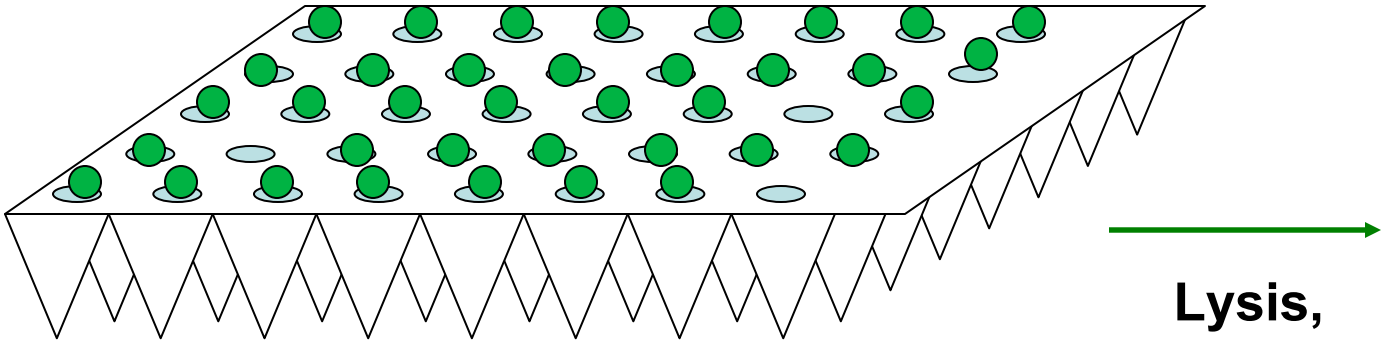
- **Dosenovic et al. (P04-28):** Use of B-cell ELISPOT to study (memory) Env-specific responses

  => Use of alternate ELISPOT setup to dissect frequency of different B cell specificities; *caveat: functionality of Abs unknown*

Sort/selection of single gp140-binding B cells

PBMCs FACS-sorted for CD19+, CD27+, IgG+ memory B cells





Lysis, RT-PCR

Conclusion

NAb field has made significant progress in dissecting (n)Ab responses in different species, particularly rabbits and humans. It is now clear that additional nAb (fine) specificities exist.

Progress has also been made in isolating new mAbs, though none as broad as the current ones. Abs to MPER region are elicited during infection.

Mucosal immmunology: application-focused. More work in process to link results to current understanding of systemic Ab responses and specificities.