the collaboration for AIDS vaccine discovery

Harnessing Innate Immunity to Enhance Immunogenicity of HIV Vaccines

<u>Objective #4</u>: Using the rhesus macaque model, ascertain the local and systemic effects of adjuvants and microbial vectors on innate/adaptive immunity and on protection from SHIV/SIV challenge.

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Global HIV Vaccine Enterprise

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The Magnitude, Breadth and Quality of Gag Responses Will be Critical for an Effective T Cell Vaccine Against HIV

- **DNA**
  - Elicit low level T cell responses to HIV Gag
- **rAd-5**
  - rAd5 HIV Gag induces ~300/10^6 IFN-g producing cells which are not protective (STEP)
- **DNA prime-rAd-5 boost**
  - Induces ~200-300/10^6 IFN-g producing HIV Gag specific cells (VRC)
- **DNA prime-MVA or NYVAC boost**
  - Induces low level Gag responses

Develop vaccine formulations and prime-boost regimens that optimize HIV Gag T cell responses

Heterologous Prime-Boost Immunization will be Required for a Successful Vaccine Against HIV

- **Primary immunization influences the magnitude and quality of T cell responses after the boost**
  - CD4+ T cells (IL-2) “programs” CD8+ responses for expansion following the boost
  - CD8+ T cell responses generated after a prime are expanded
- **STEP Trial using rAd-5 Gag/Pol/Nef showed that <u>only 33%</u> of vaccines had both CD4 and CD8+ T cell responses**

Vaccine Platforms to Optimize Gag Specific T Cell Responses

- **Prime**
  - **DNA**- Must improve delivery (electroporation)
  - **rAd-35 or 26**-Are more efficient than DNA in terms of the number of immunizations needed for priming. Prior use as vaccines early in life for other infections (TB, Malaria) may limit their immunogenicity
  - **Pox (MVA or NYVAC)**-Have been used as a boost following DNA most current clinical trials.
  - **Protein**

Rationale for Protein Based Vaccines

- Protein vaccines induce broad-based immune responses

  -Antibody

  -Th1 *and under certain conditions* CD8+ T cell responses

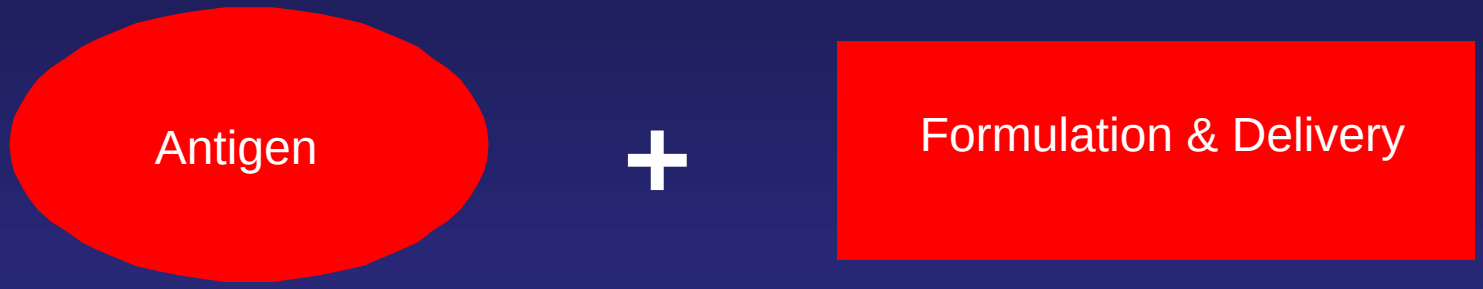
- Protein vaccines are not limited by pre-existing immunity

  -Use as a prime prior to viral vector

  -Use as a boost to augment antibody or T cell responses

  -Use to maintain or enhance antibody or T cell memory

Components of a Vaccine

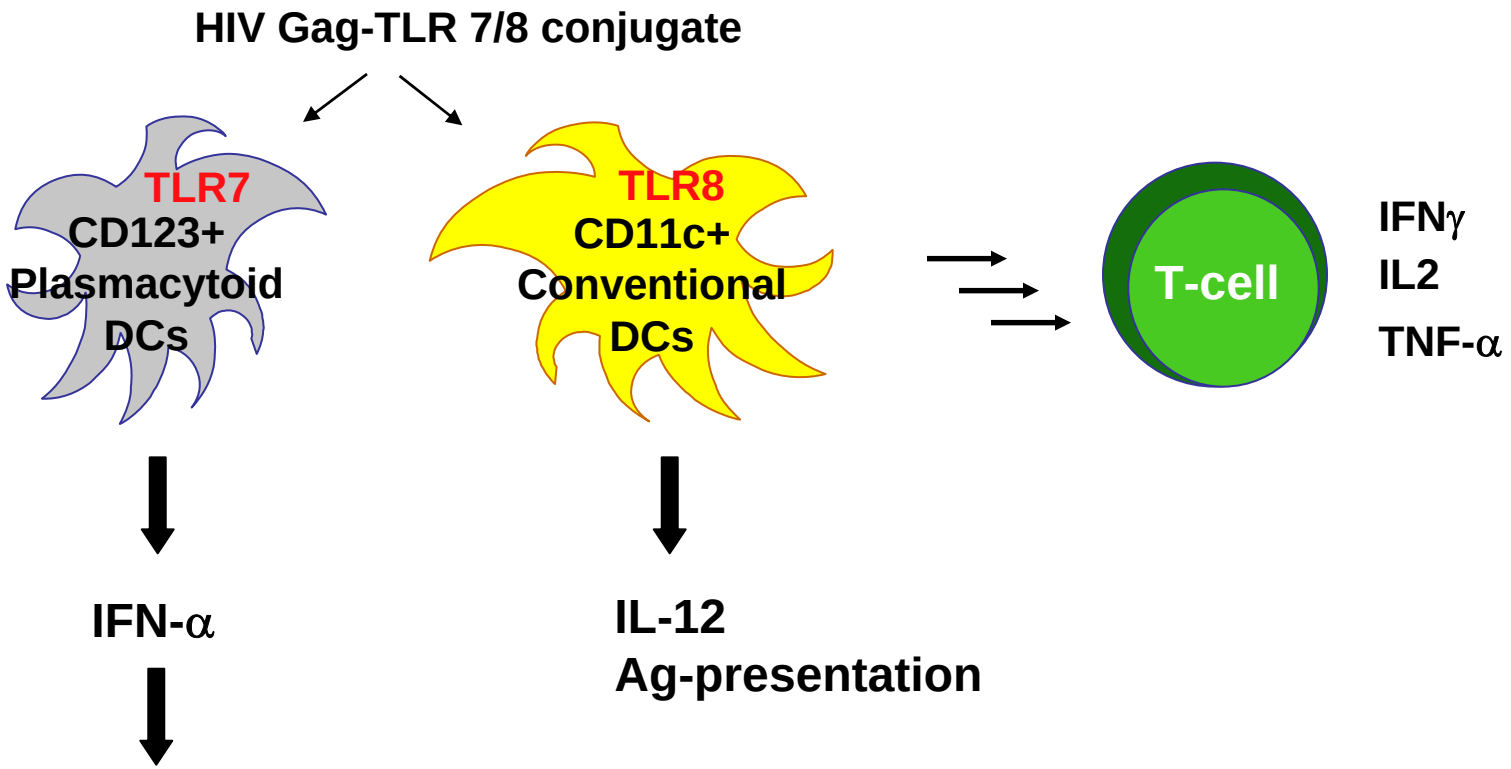


Adjuvant/conjugate
Vaccine vehicle

Specificity

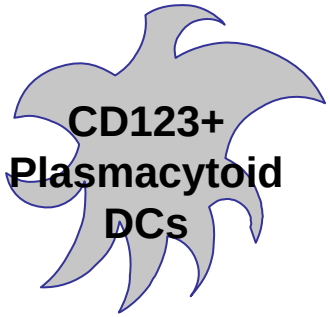
Magnitude
Composition
Duration
Compartmentalization

Activation of Dendritic Cells is Critical for Induction of Multi-functional Th1 and CD8 T Cell Responses



-Increase Th1 responses
-Required for cross-presentation with protein vaccine
-Enhance CD8 T cell expansion

TLR Ligands Activate Human Dendritic Cells



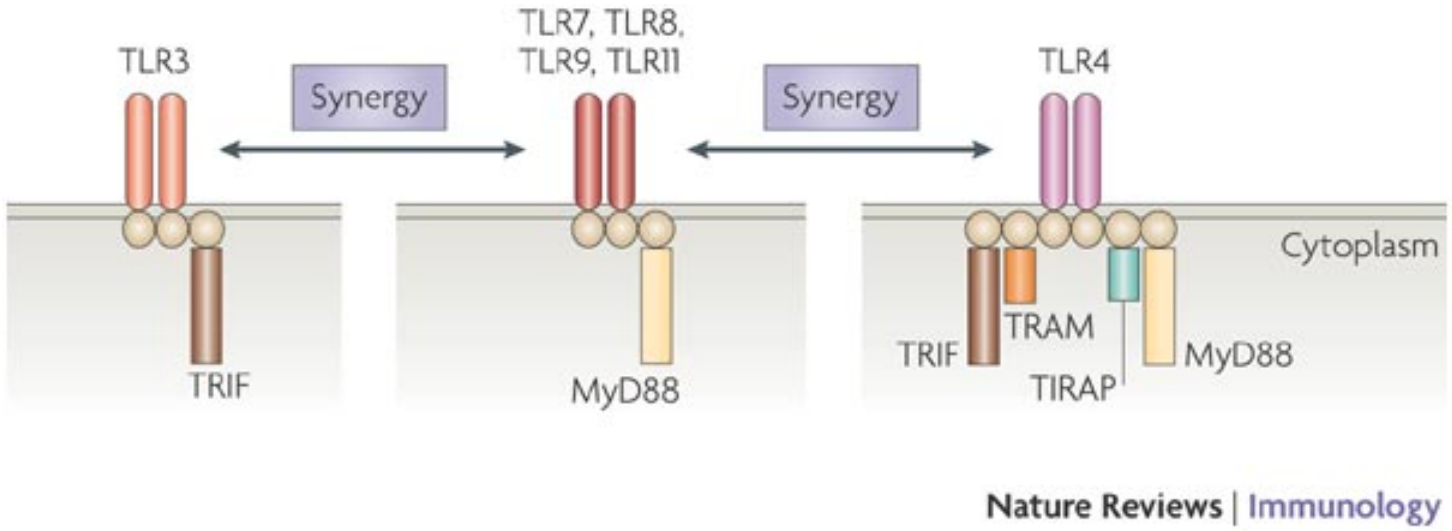
Ag presentation

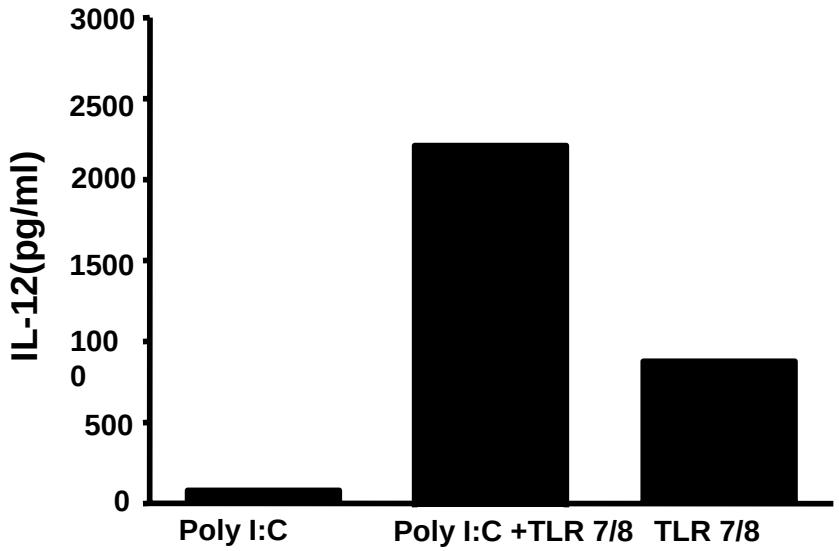
Cytokine Production:	IFN-α	IL-12	
TLR expression:			**TLR ligand:**
TLR 3	-	+	dsRNA (Poly I:C)*
TLR 4	-	+	LPS (MPL)
TLR 7/8	+	+	ssRNA (TLR7/8L)
TLR 9	+	-	CpG

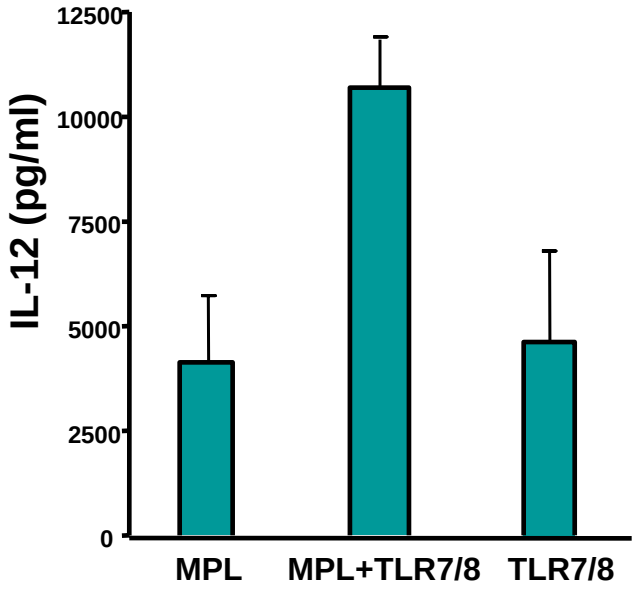
*Poly I:C can induce IFN-α via TLR independent pathways (RIG-I, MDA-5)

Signaling Pathways for TLR Synergy

Trinchieri and Sher *Nature Reviews Immunology* **7**, 179–190





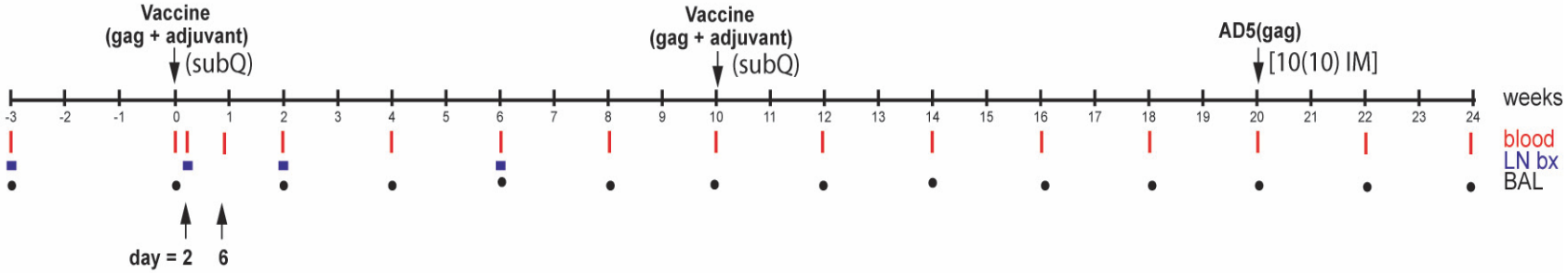


Goal of Studies

- Compare the ability of TLR3 (Poly I:C*), TLR 4 (MPL), TLR 7/8 (3M-012) and TLR 9 (CpG) ligands to generate SIV gag immunity when administered with Montanide ISA 51
  - Montanide ISA 51 is an oil/water emulsion that creates depot
- Determine whether combinations of TLR ligands enhance immunity
  - Signaling synergy (MyD88 and Trif) on the same dendritic cell
    - TLR 3 + TLR 7/8
    - TLR 4 + TLR 7/8
  - Activation of cDCs and pDC by distinct TLR ligands
    - TLR3 (cDC) + TLR 9 (pDC)
    - TLR4 (cDC) + TLR 9 (pDC)

Experimental Protocol

NHP Adjuvant Experiment # 1



Groups (all vaccines prepared in montanide ISA 51 carrier):

1. SIVgag
2. SIVgag + TLR3
3. SIVgag + TLR4
4. SIVgag + TLR7/8
5. SIVgag + TLR9
6. SIVgag + TLR3 +TLR7/8
7. SIVgag + TLR3 + TLR9
8. SIVgag + TLR4 + TLR7/8
9. SIVgag + TLR4 + TLR9
10. no protein (Ad5-gag only)

4RM per group with each RM getting the same vaccine preparation at time = 0 and 10 wks.

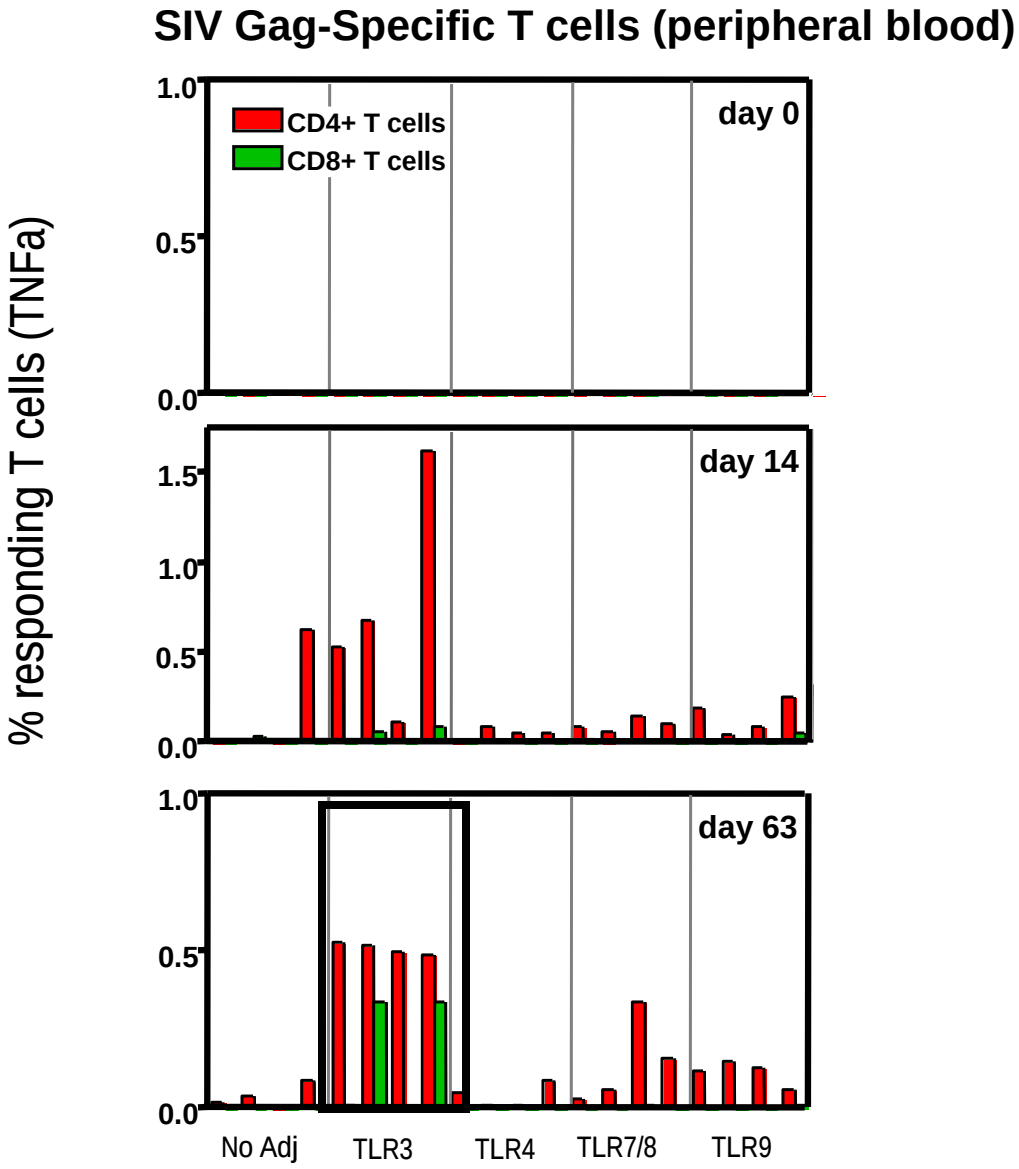
SIVgag = SIVmac239 gag p55
TLR3 = poly I:C
TLR4 = MPL
TLR7/8 = 3M-012
TLR9 = CpG

Challenge with $SIVmac_{251}$ IV @ 210 days post Ad-5 Gag boost.

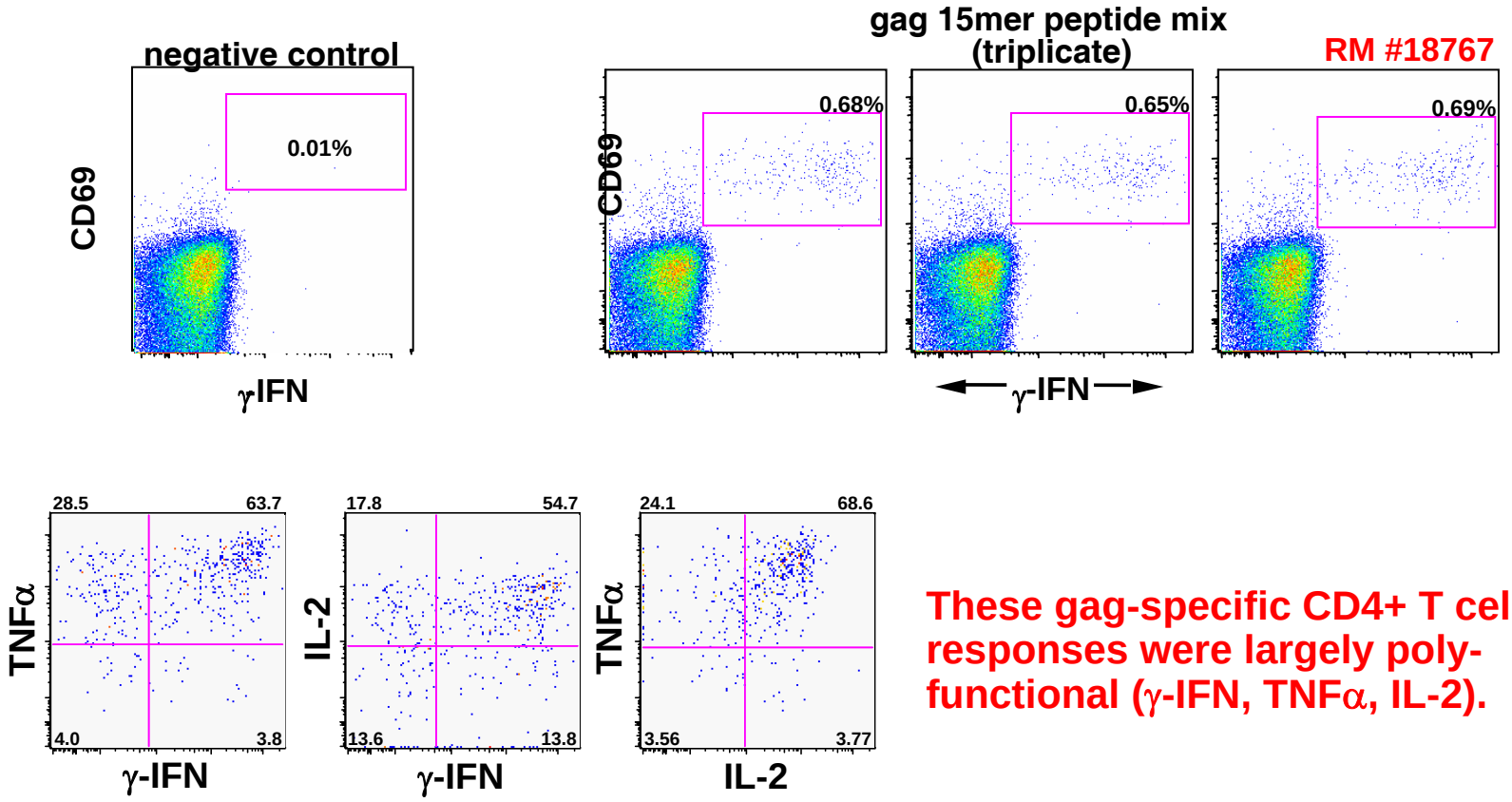
Analyses

- SIV gag-specific T cell responses (Blood, BAL, LN)
  - Cytokine flow cytometry (γ-IFN, TNF, IL-2) using 15mers peptide mixes
  - CM9 Tetramer (in A*01+ RM) to assess dominant CD8 responses
- SIV gag-specific antibody responses (plasma)
- Phenotypic analysis (blood, LN)
  - T cells, B cells, NK cells, dendritic cells
- Gene array analysis (PBMC, LN)

Poly I:C (TLR3) is the Most Effective Single TLR Adjuvant for Eliciting Th1 *and* CD8+ T Cell Responses



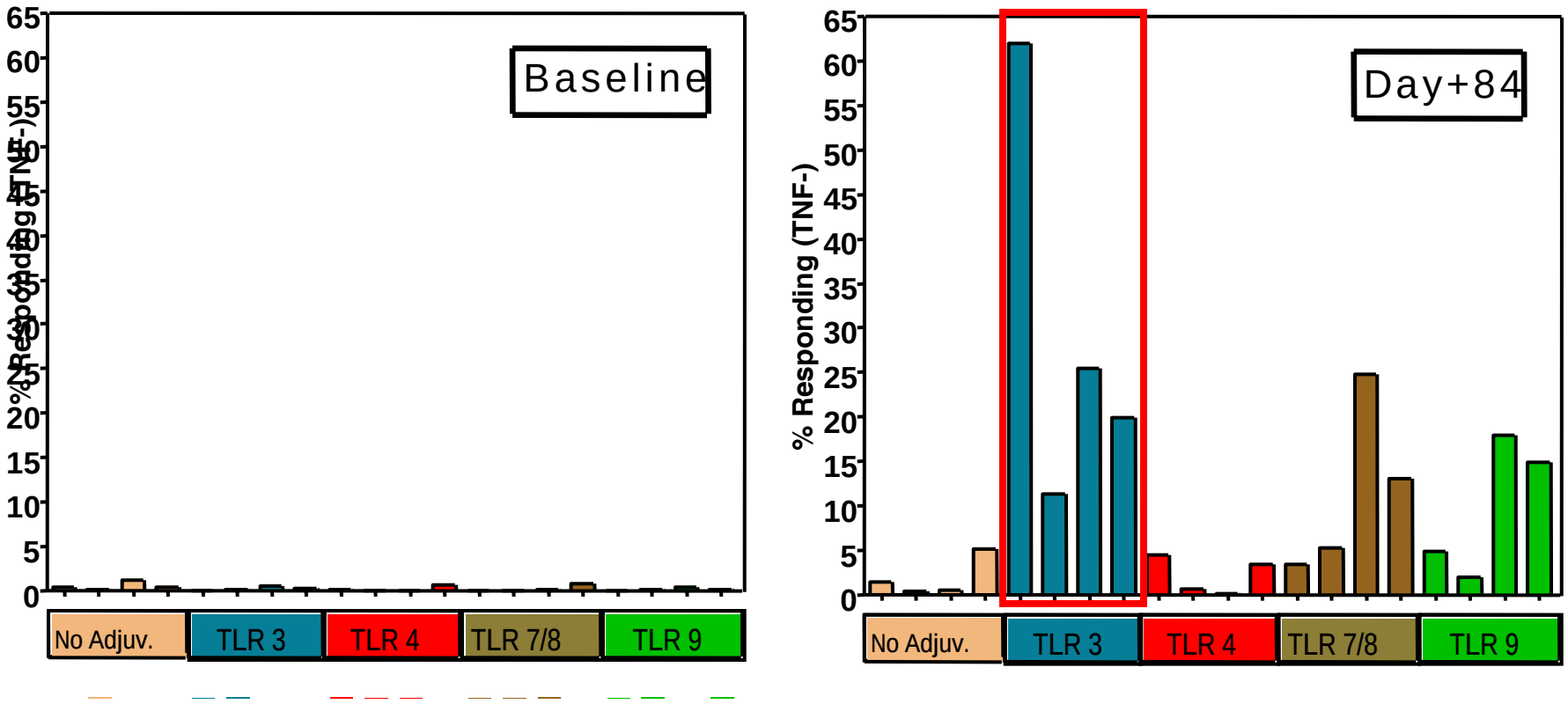
SIV Gag+Poly I:C Elicited Multi-Functional CD4+ T Cell Cytokine Responses



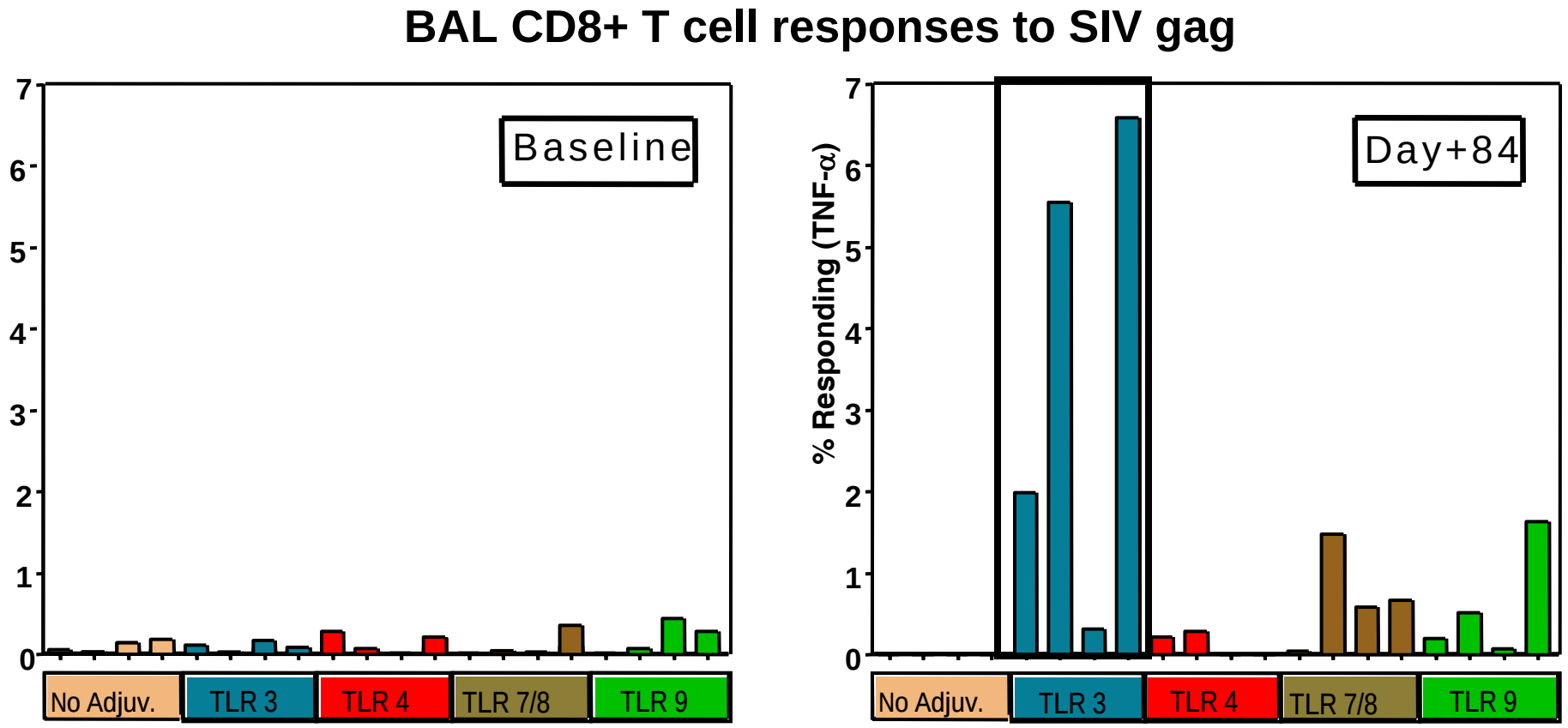
These gag-specific CD4+ T cell responses were largely poly-functional (γ-IFN, TNFα, IL-2).

“Double Digit” CD4+ T Cell Cytokine Responses were Induced in BAL in Monkeys Immunized with Poly I:C

BAL CD4+ T cell responses to SIV gag

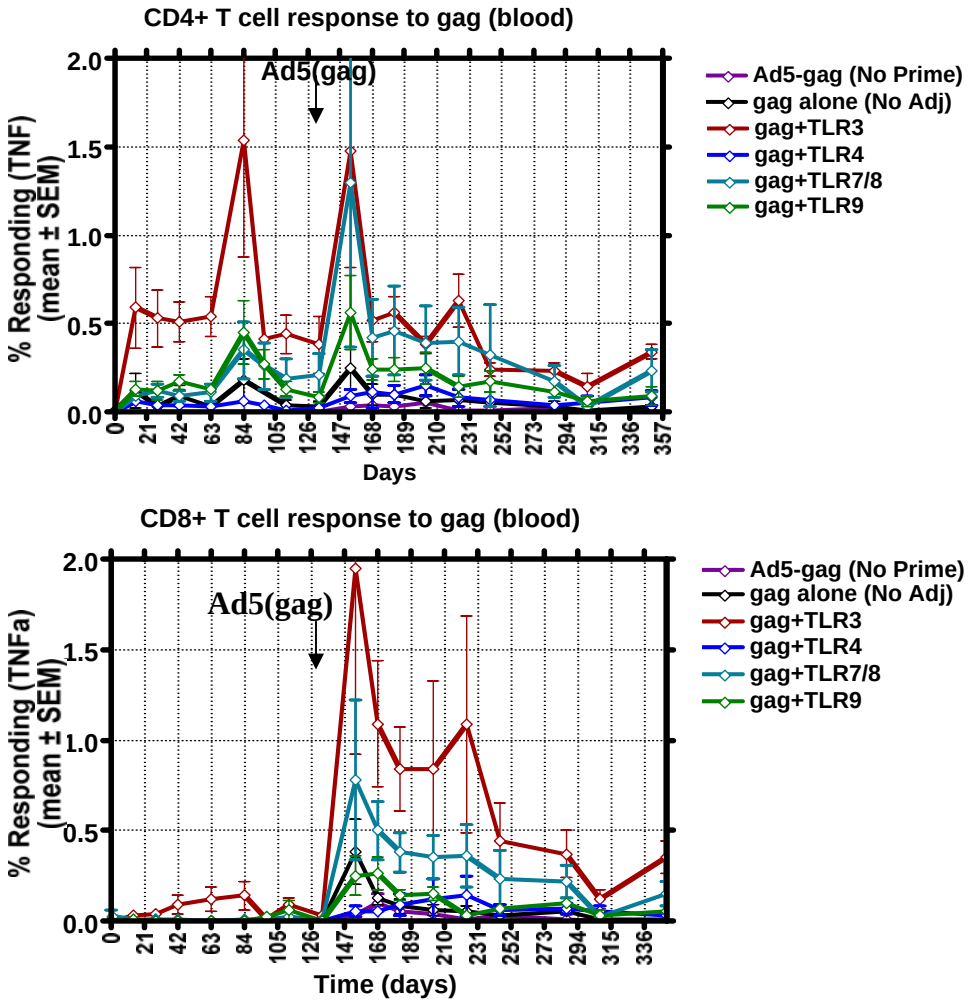


Gag-Specific CD8+ T Cell Responses in BAL were Highest in Monkeys Immunized with Poly I:C



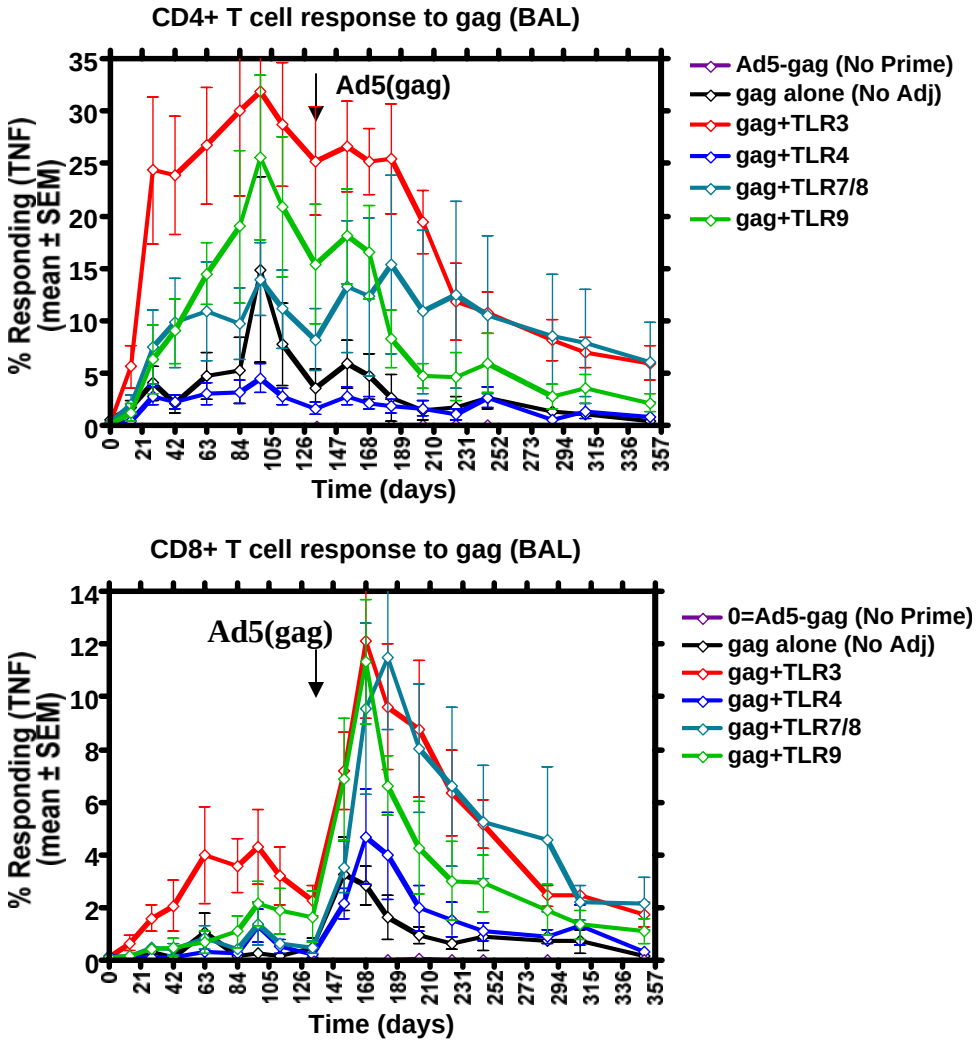
Poly I:C (TLR 3) and TLR 7/8L are the Most Efficient Enhancers of Long-Term SIV Gag T Cell Responses in Blood

Blood

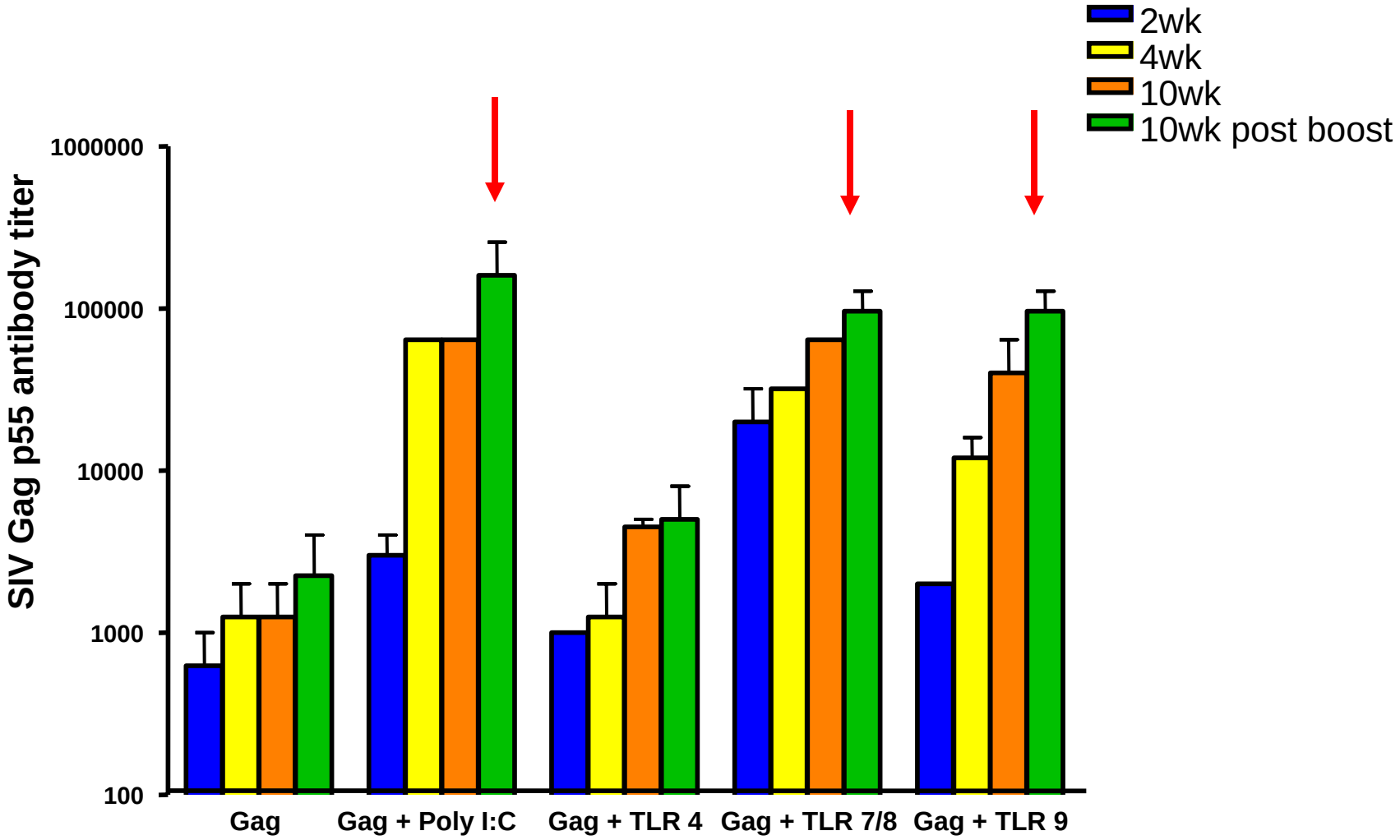


Poly I:C (TLR 3) and TLR 7/8L are the Most Efficient Enhancers of Long-Term SIV Gag T Cell Responses in BAL

BAL



Gag-specific Antibody Responses were Substantially Enhanced by Poly I:C, TLR7/8 and TLR9 agonists



Conclusions

- Protein-based vaccines can elicit potent CD4+ *and some* CD8+ T cell responses when formulated with certain TLR agonists
- Poly I:C and TLR 7/8 are the most potent of the TLR agonists studied
- T cell responses elicited by these protein + adjuvant vaccines accumulated to high frequency in effector sites (BAL)
- No synergy was noted with combination of TLR agonists
- Increased breadth of CD8+ Gag responses was noted in animals that received Poly I:C

Protein and Poly I:C is promising approach for optimizing humoral and cellular responses