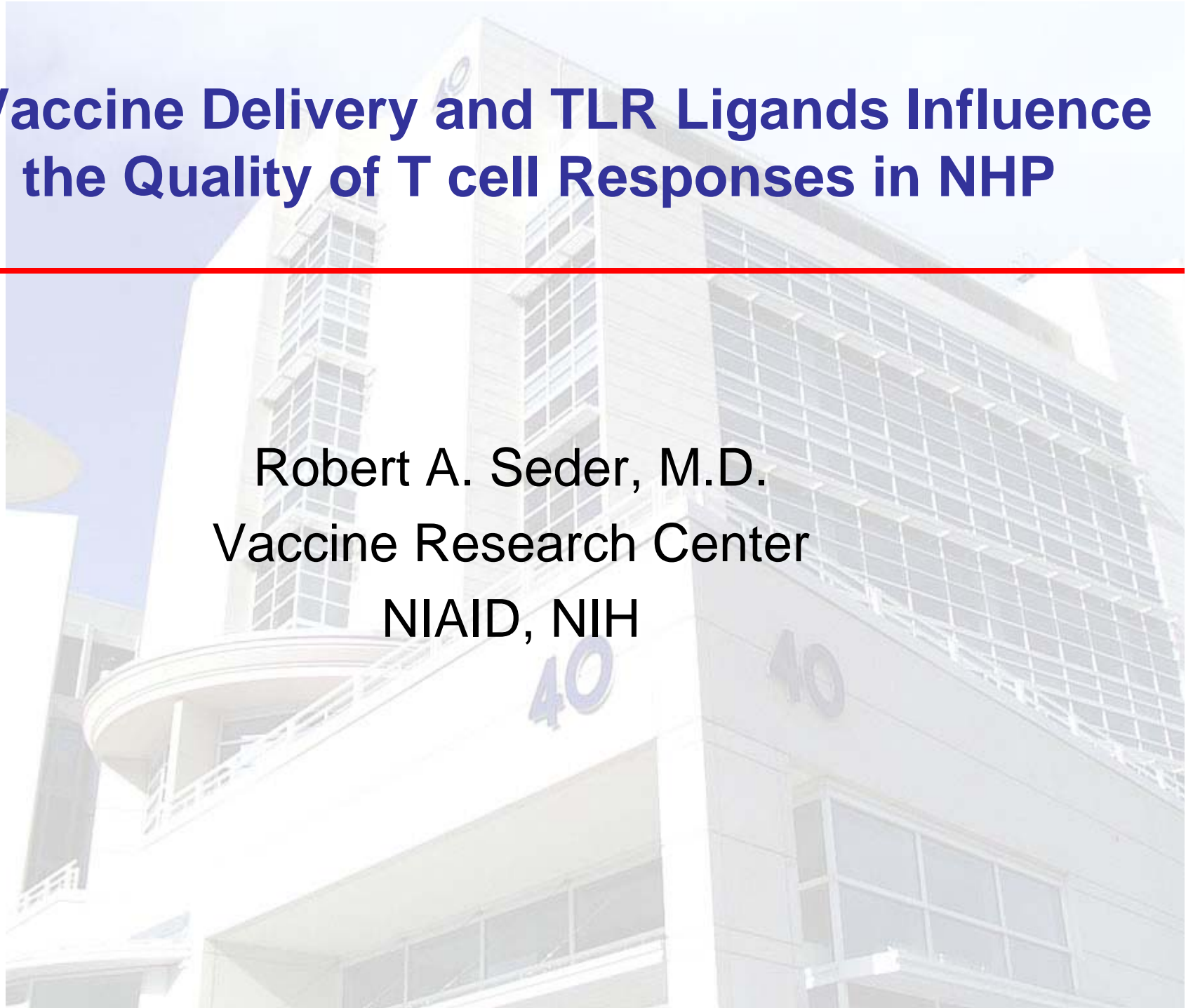


Vaccine Delivery and TLR Ligands Influence the Quality of T cell Responses in NHP

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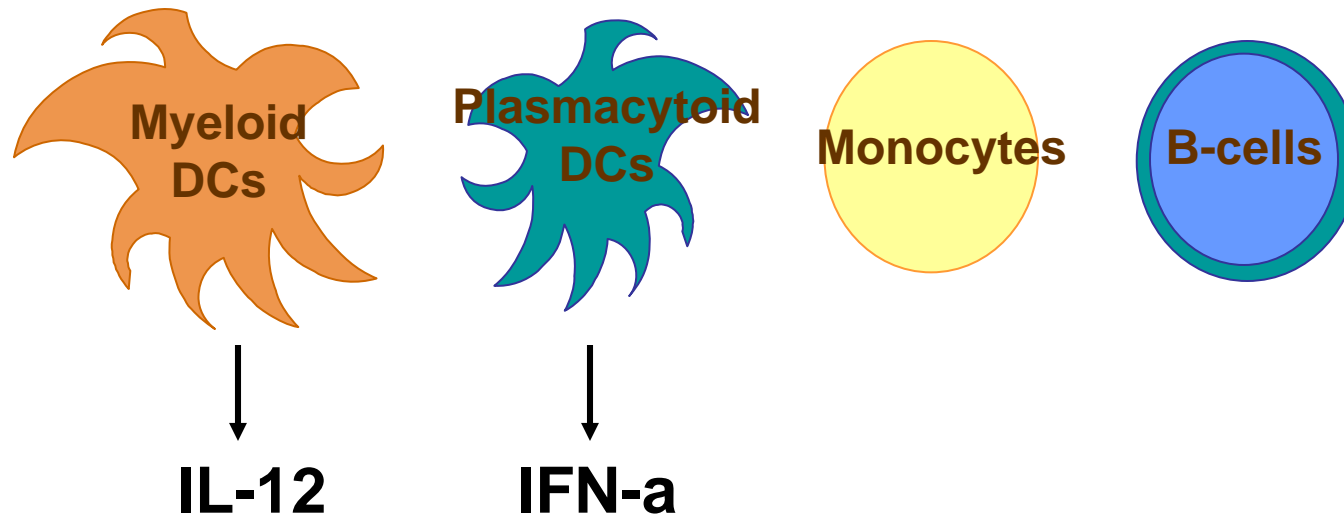
Vaccines Against HIV, Malaria and Tuberculosis Will Require Neutralizing Antibody and Cell-Mediated Immunity

- Design vaccines that elicit broad-based immunity
- Develop methods to characterize better T cell responses
- Define a T cell response that will be optimal for mediating protection

Rational for protein based vaccines

- **Protein vaccines induce broad-based immune responses**
 - **Antibody**
 - **Th1 *and* CD8+ T cell responses**
- **Protein vaccines are not limited by pre-existing immunity**
 - **Used to induce or enhance T cell responses**
- **Vaccine formulation and the type of TLR ligand are important variables for optimizing responses**

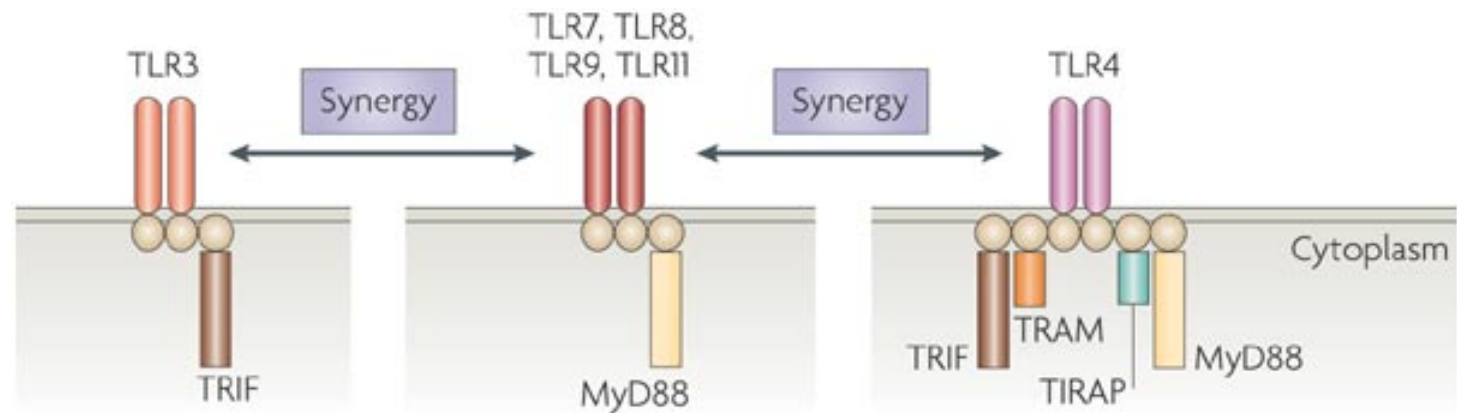
Toll like receptor expression in antigen presenting cells



TLR expression

TLR 4	++	-	++	+
TLR 7	-	++	-	+
TLR 8	++	-	++	-

Signaling Pathways for TLR Synergy



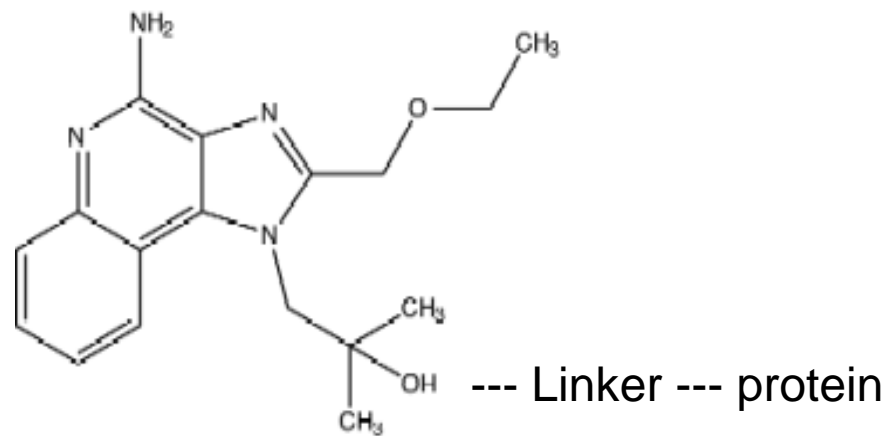
Nature Reviews | Immunology

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

Napolatani et al. *Nature Immunology* 6, 769 - 776 (2005)

Vaccine Formulation

- Oil/water (SE), Liposomes, ISCOMS, Montanide
- Conjugation of protein to TLR 7/8 ligand
 - HIV Gag-TLR 7/8 conjugate induces potent antibody and multi-functional Th1 *and* CD8+ T cell responses in non-human primates



Goal of Studies

- Compare the magnitude and quality of T cell responses in non-human primates varying the vaccine formulation and the type of TLR ligand
- **Formulation**
 - Administer protein with TLR 7/8 ligand in oil/water emulsion (SE)
 - Protein conjugated to the TLR 7/8 ligand
- **Determine whether combining TLR ligands elicit higher responses**
 - Compare TLR 4 (MPL)+ TLR7/8 ligand vs MPL or TLR 7/8 alone

Immunization Protocol

Vaccine Groups

1. MML protein-TLR 7/8 conjugate
2. MML protein+TLR 7/8 SE
3. MML protein+ MPL/SE
4. MML protein+ MPL/SE+TLR 7/8

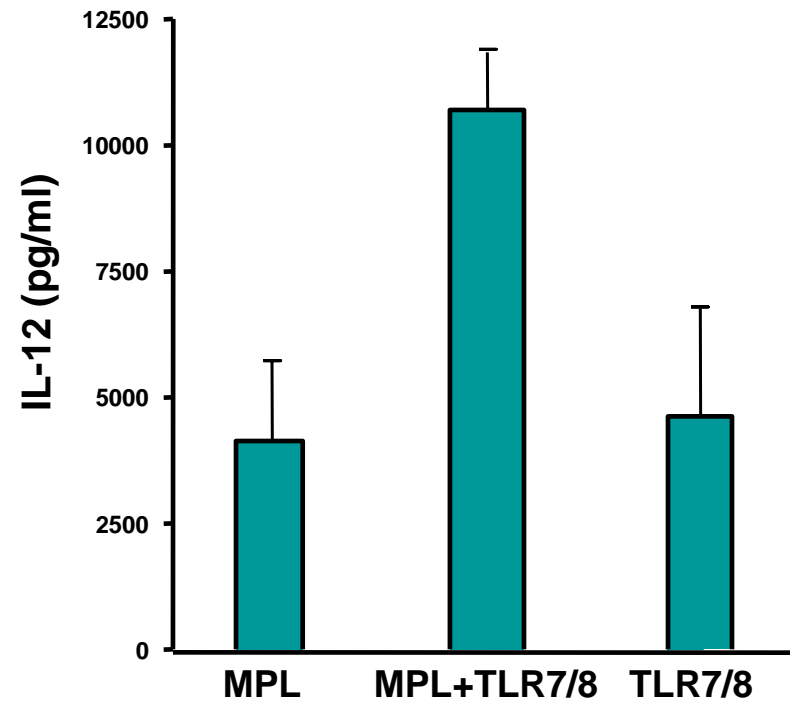
-Immunization: 0, 1 and 3 months

-Analysis of T cell responses:

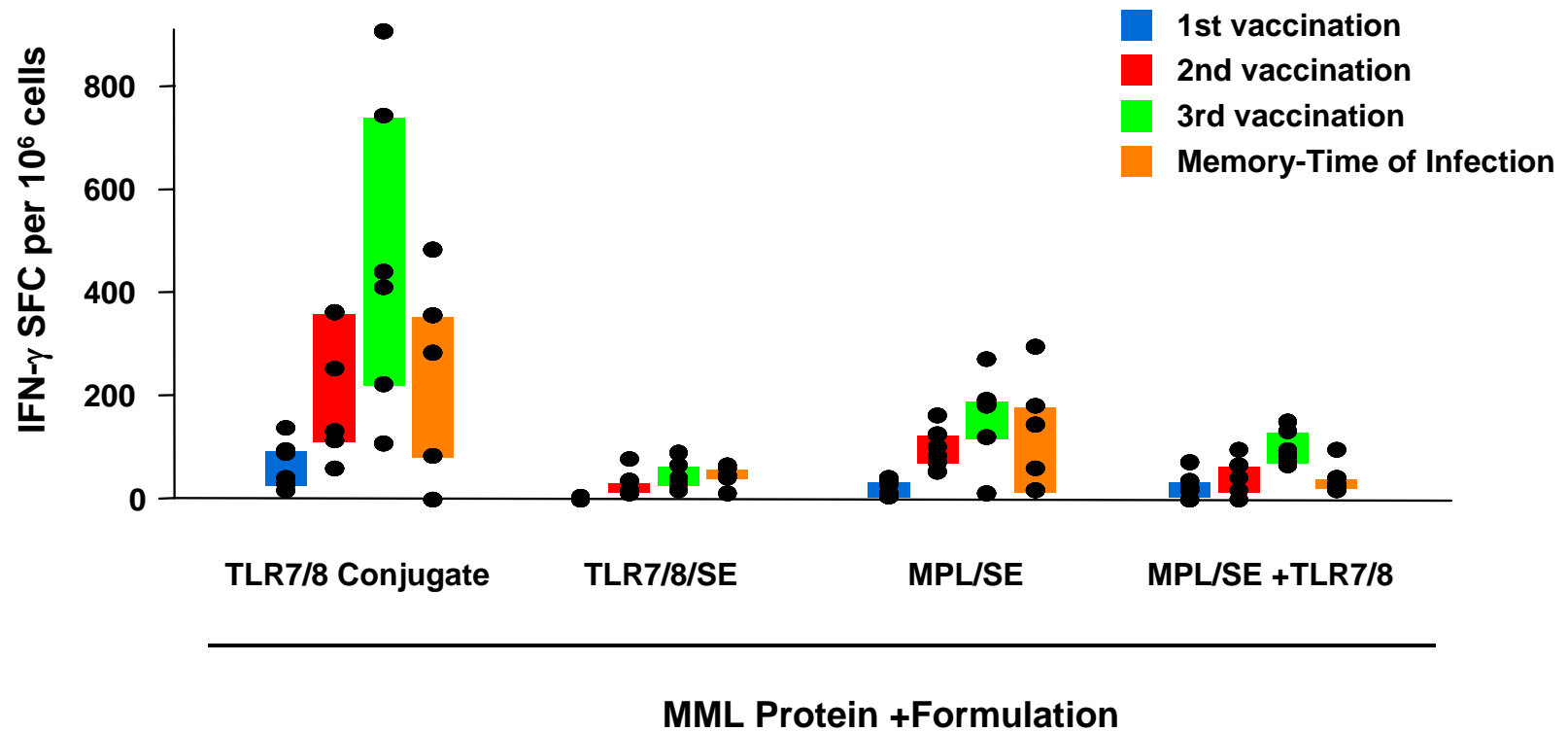
Magnitude-Frequency of IFN- γ producing T cells by Elispot

Quality-Assess T cell response by multi-parameter flow cytometry

MPL+TLR 7/8 ligand induces more IL-12 from monkey PBMCs than either TLR alone



MML-TLR 7/8 conjugate induces the highest frequency of IFN- γ producing cells



Defining the quality of the response with multi-parameter flow cytometry

Staining panel

Dead cells (ViViD)

CD3 (PerCP Cy5.5)

CD4 (Alexa 700)

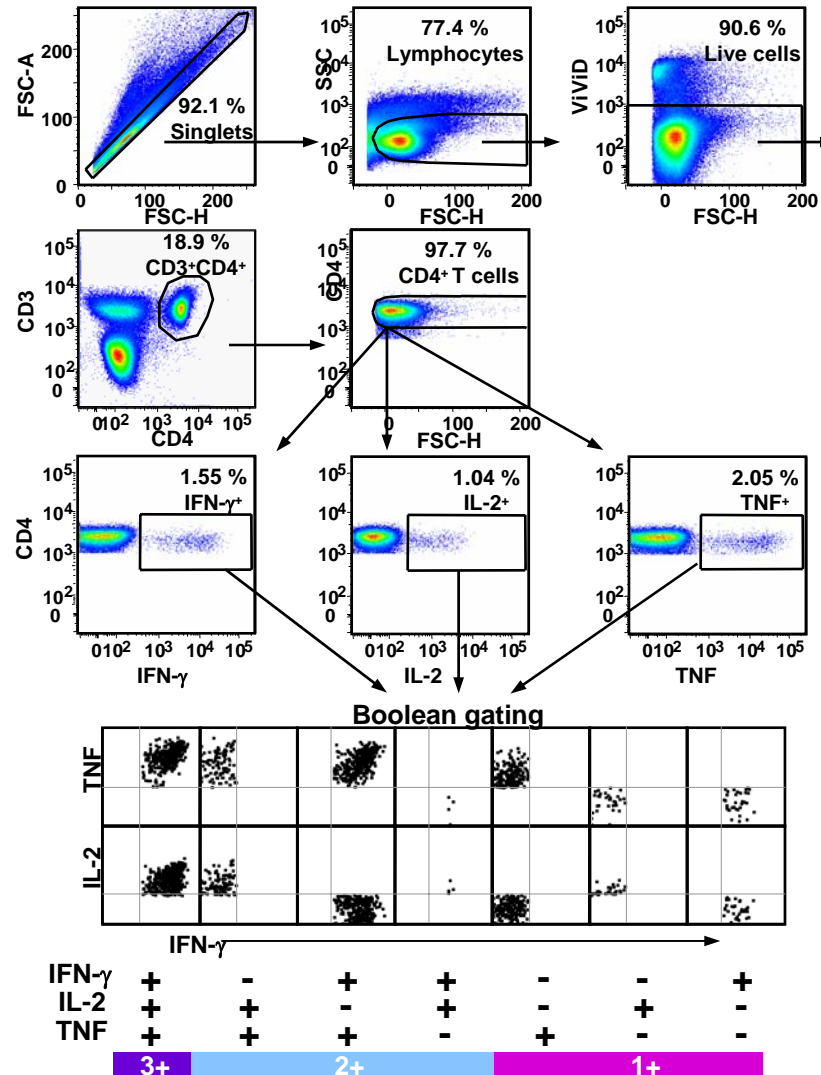
CD8 (APC Cy7)

CCR7 (Biotin / QD 655 SA)

IFN- γ (APC)

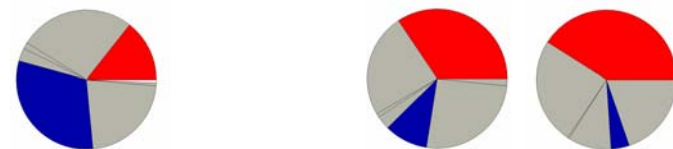
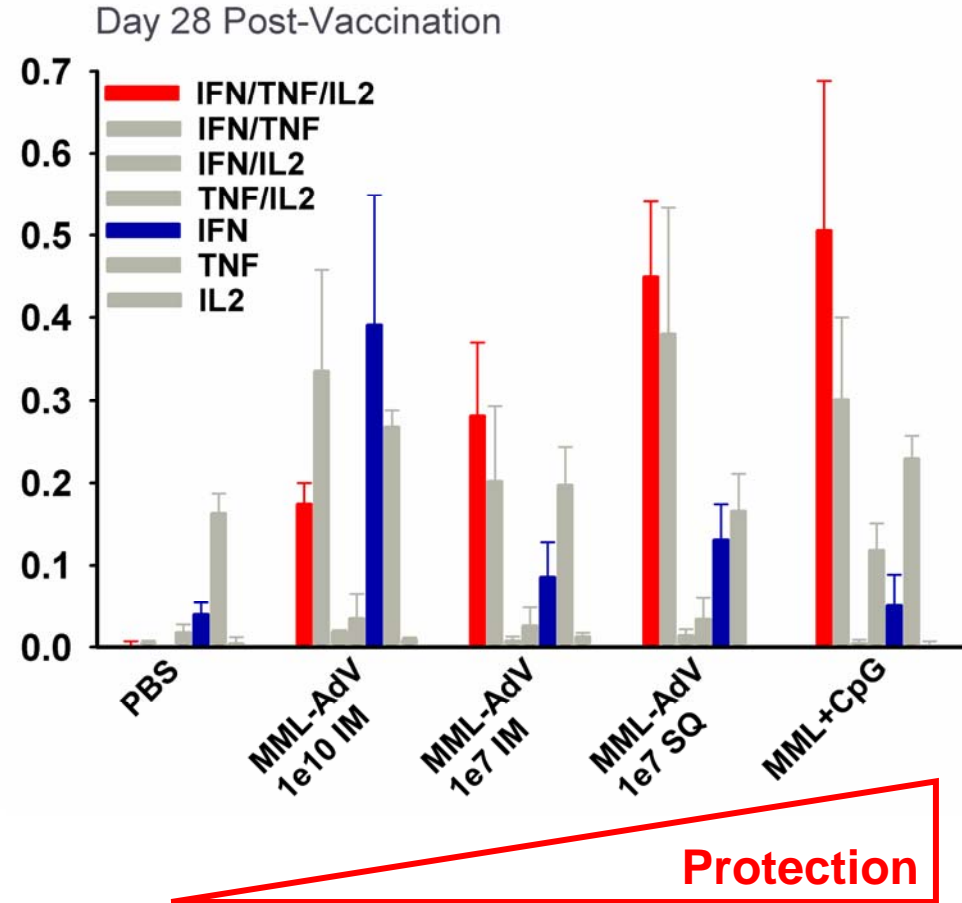
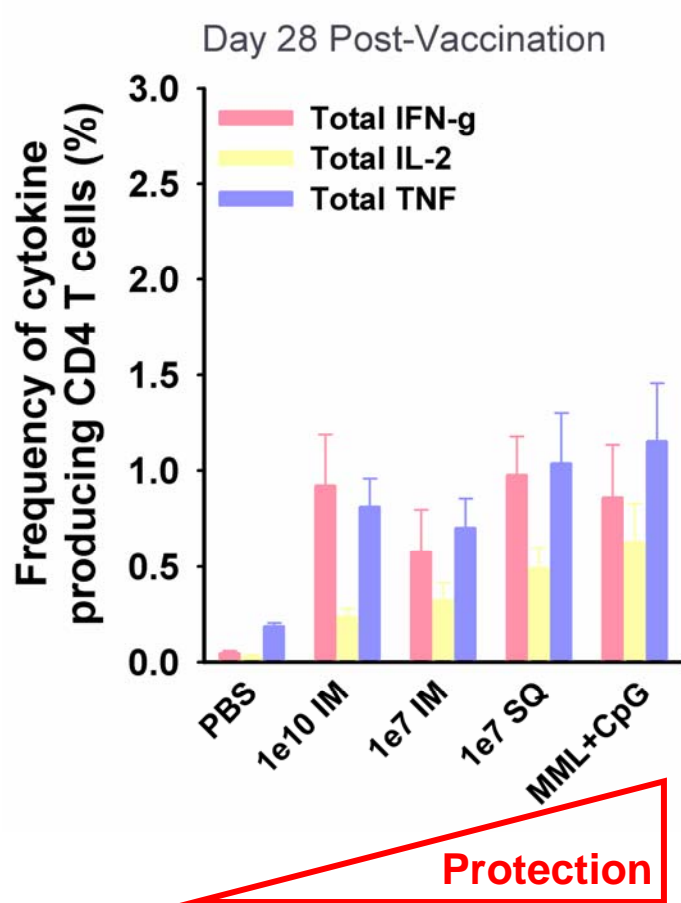
IL-2 (PE)

TNF (PE Cy7)



Fraction of response

Multi-Functional Th1 Responses (IFN- γ +IL-2+TNF+) Correlate With Protection

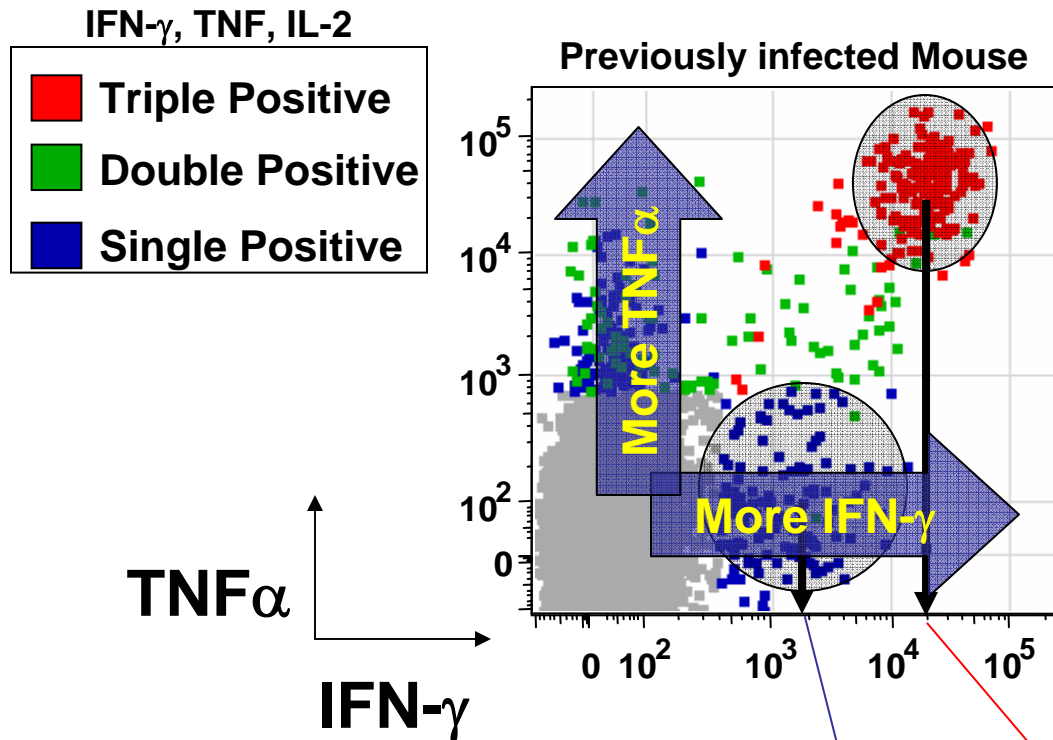


Percent of total response

Why are multi-functional cells better?

- Secretion of all three cytokines
 - Increased effector function by secreting IFN- γ and TNF α
 - IL-2 production leads to expansion of cells
- Secrete more cytokine per cell

Multi-functional Th1 cells produce more IFN- γ and TNF α



IFN- γ MFI of Single Positives: ~2,000

IFN- γ MFI of Triple Positives: ~20,000

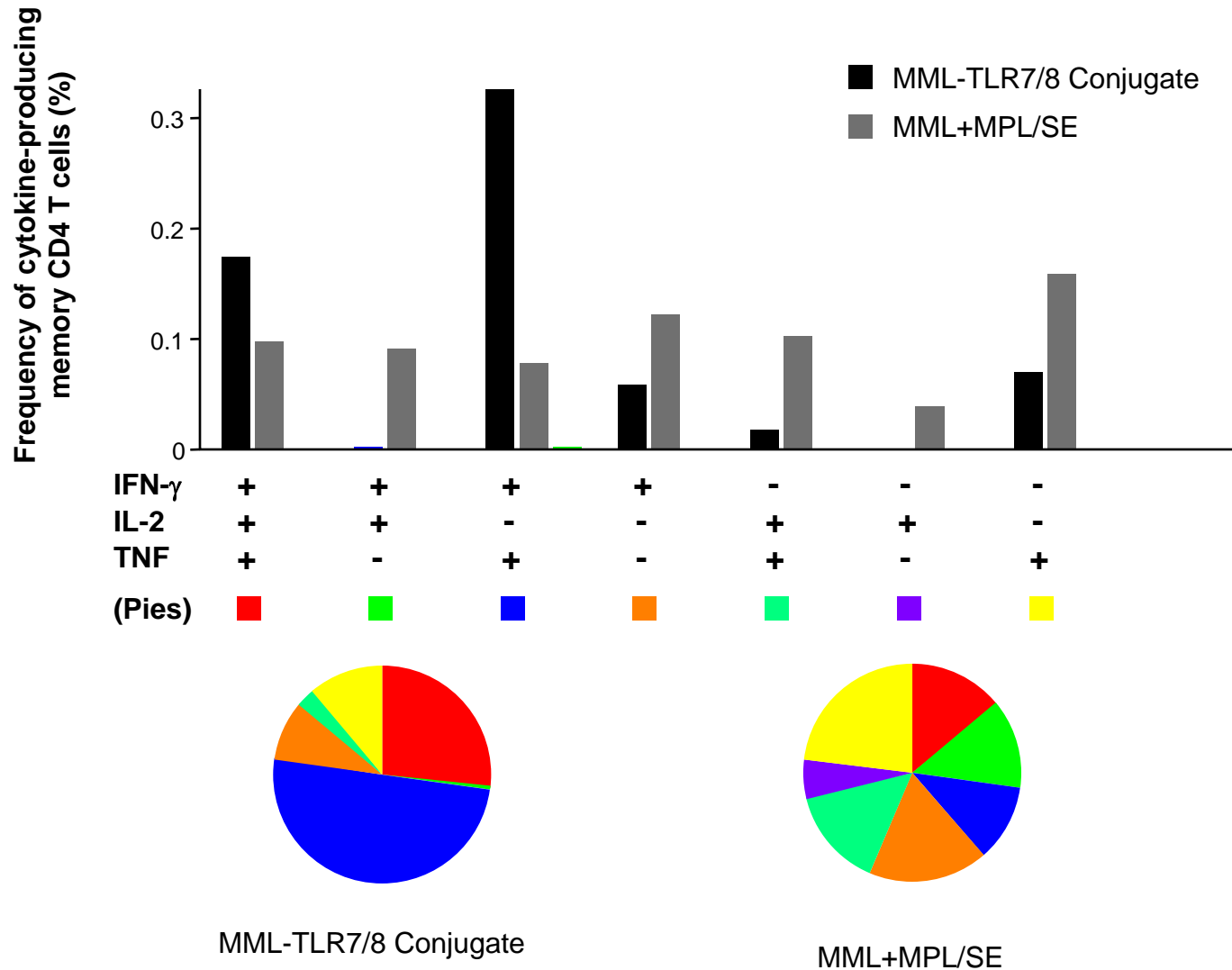
The fluorescence intensity of the cells is related to how much cytokine they have produced.

Triple-positive cells appear to produce considerably more IFN- γ and TNF *per cell*.

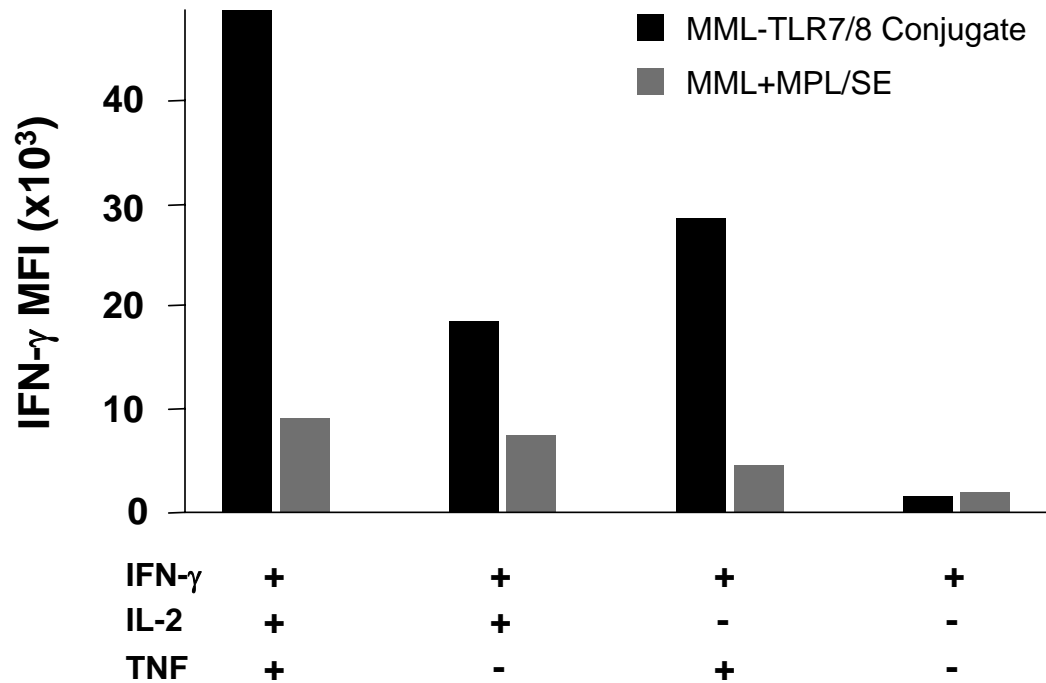
Cytokine production by a population can be estimated by the MFI (median fluorescence intensity)

Each triple positive cell is binding 10x as much anti-IFN- γ antibody as each single positive... indicating that they are capable of secreting 10x as much cytokine on a *per cell* basis.

MML-TLR 7/8 conjugate and MML+MPL/SE elicit distinctly different qualitative responses



MML-TLR 7/8 conjugate vaccines elicits more potent IFN- γ producing cells than MML+MPL/SE

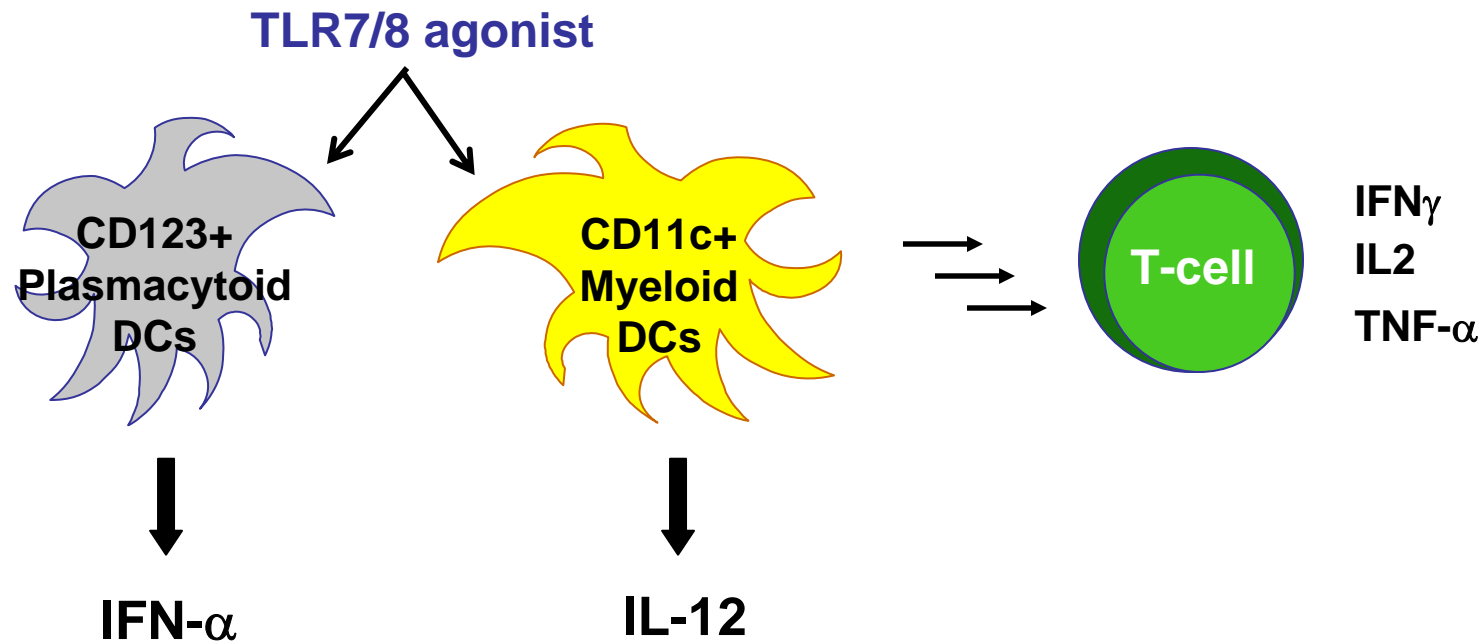


Summary

- **Protein-TLR 7/8 conjugate immunization elicits potent *and* durable T cell responses in non-human primates**
 - **Magnitude and quality of T cell responses are better than vaccines using TLR 4 or TLR 7/8 alone or in combination using SE as a vehicle**
 - **No “synergy” *in vivo* with MPL and TLR7/8 ligand**

Vaccine formulation and the type of TLR ligand are important variables for optimizing both the magnitude and quality of the T cell responses

How does the protein-TLR 7/8 conjugate vaccine elicit multi-functional T cell responses?



Which DC subsets are required for induction of Th1 and CD8 T cell responses and imprint the quality of the T cell response?

Acknowledgments

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