

A Heterologous rMuVgag/rVSVgag Prime-Boost Vaccination Regimen Elicits a Very Robust Gag-Specific Cellular Immune Response in Rhesus Macaques

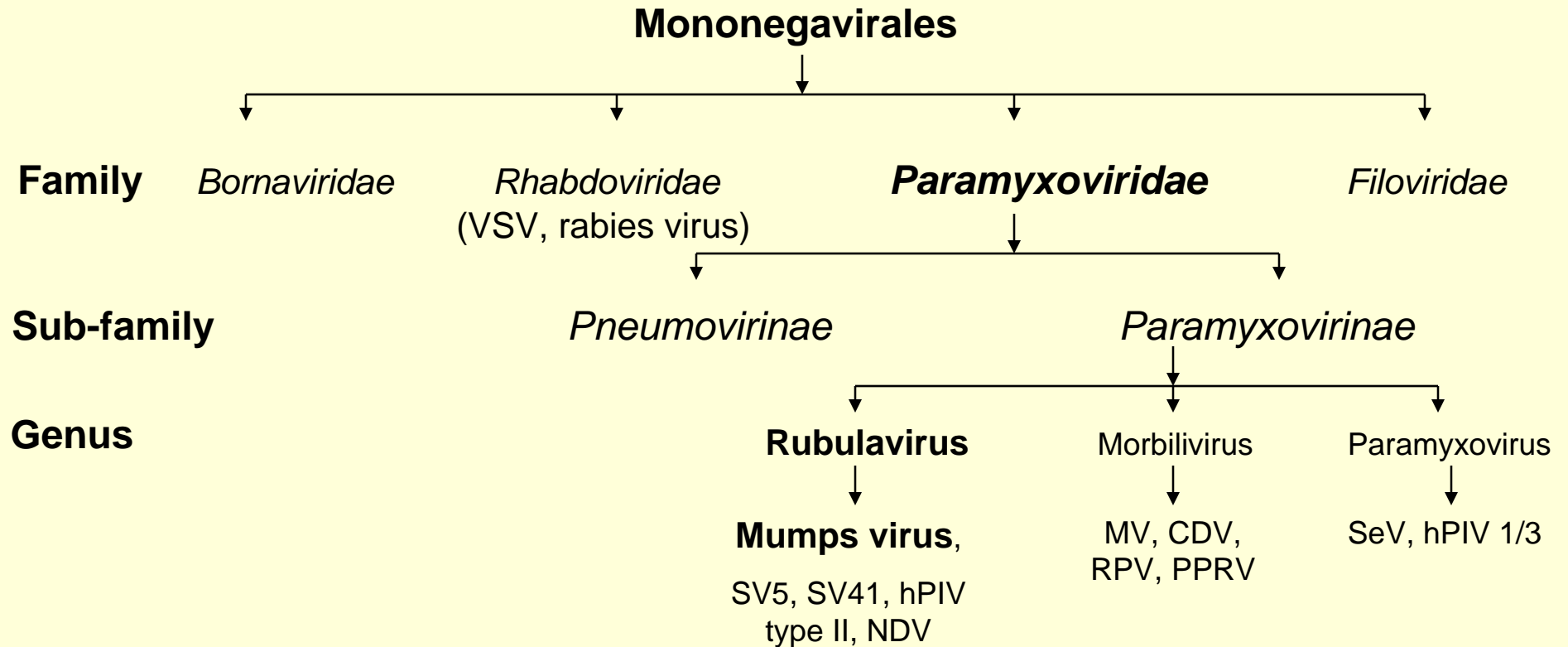
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David K Clarke

Wyeth
Research

Classification of Mumps Virus

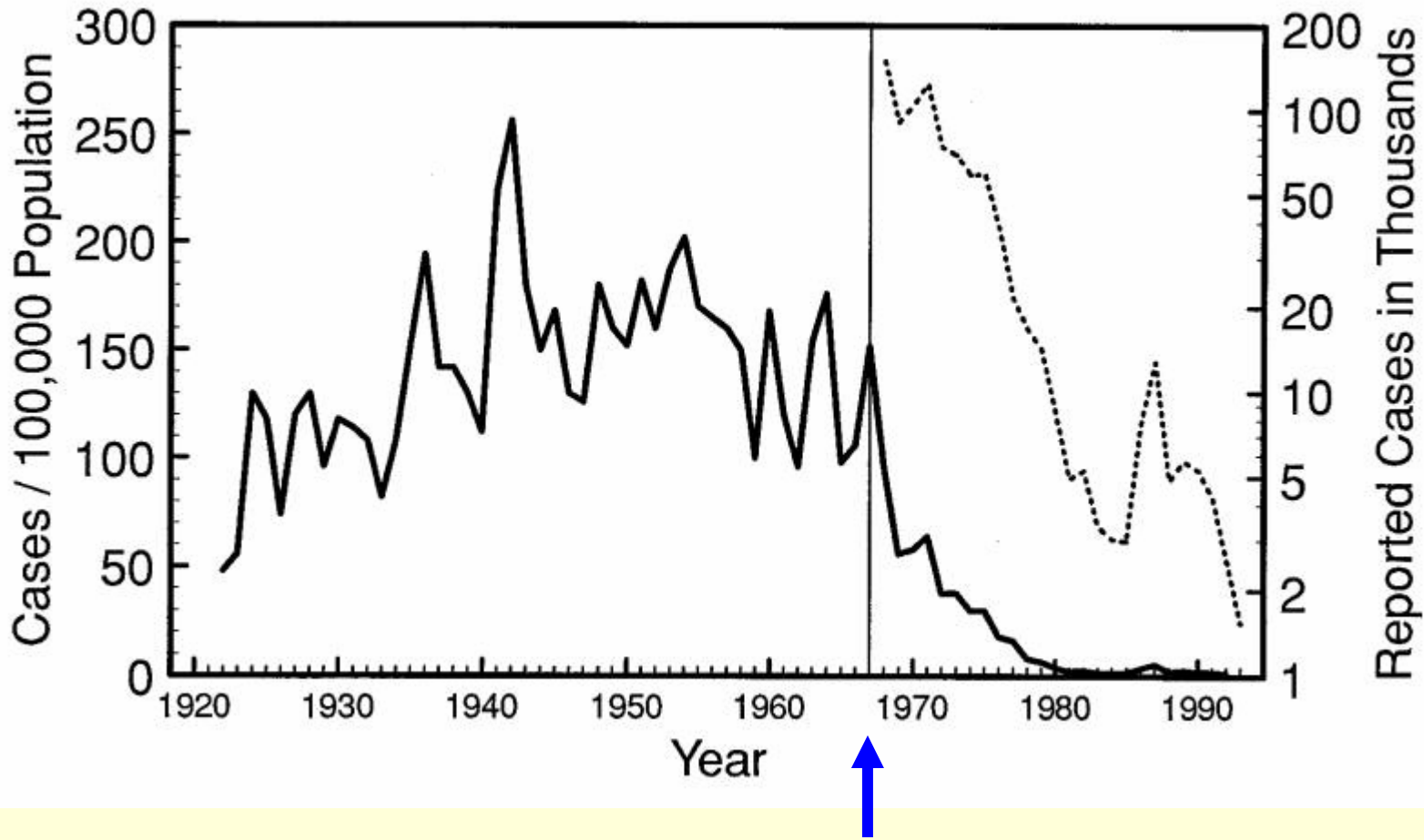


Classic Signs of Mumps Virus Infection



Mumps: Note the bilateral parotid and submandibular gland enlargement.

Incidence of Mumps in the USA



Rescue Of Mumps Virus

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Rescue of Mumps Virus from cDNA

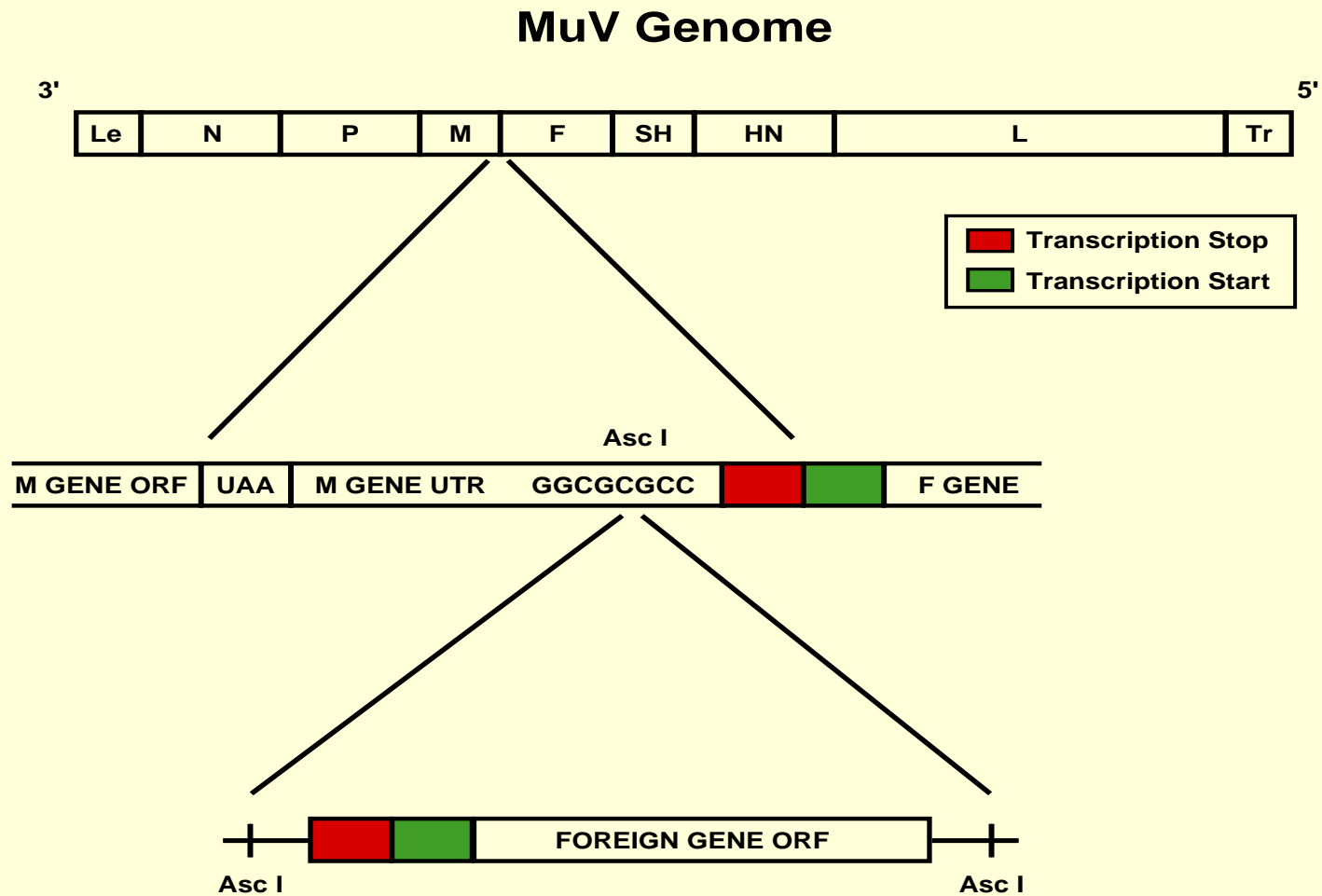
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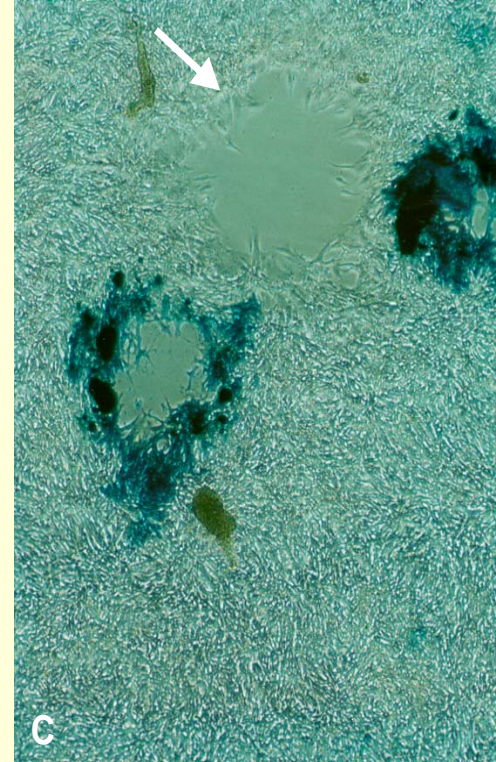
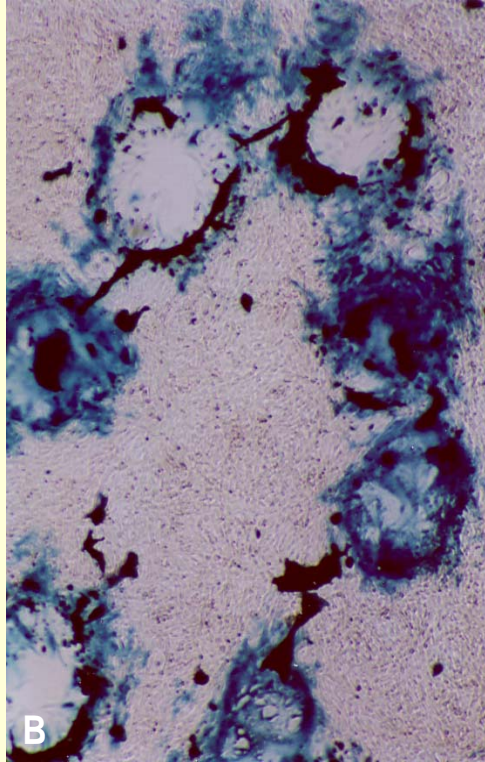
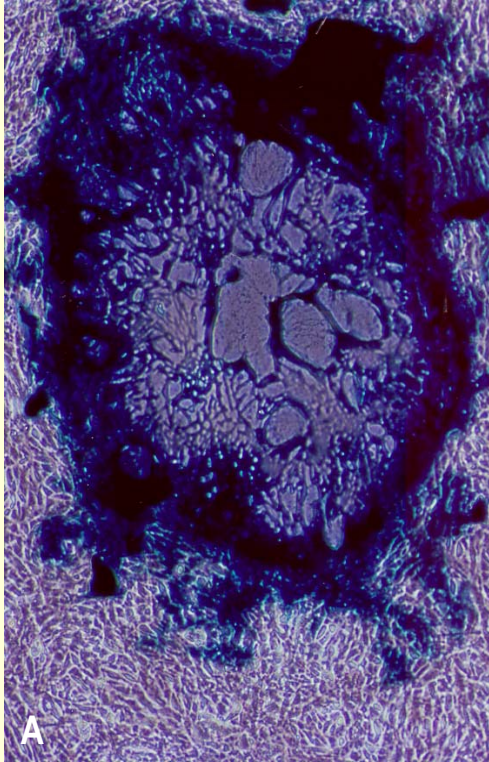
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A complete DNA copy of the genome of a Jeryl Lynn strain of mumps virus (15,384 nucleotides) was assembled from cDNA fragments such that an exact antigenome RNA could be generated following transcription by T7 RNA polymerase and cleavage by hepatitis delta virus ribozyme. The plasmid containing the genome sequence, together with support plasmids which express mumps virus NP, P, and L proteins under control of the T7 RNA polymerase promoter, were transfected into A549 cells previously infected with recombinant vaccinia virus (MVA-T7) that expressed T7 RNA polymerase. Rescue of infectious virus from the genome cDNA was demonstrated by amplification of mumps virus from transfected-cell cultures and by subsequent consensus sequencing of reverse transcription-PCR products generated from infected-cell RNA to verify the presence of specific nucleotide tags introduced into the genome cDNA clone. The only coding change (position 8502, A to G) in the cDNA clone relative to the consensus sequence of the Jeryl Lynn plaque isolate from which it was derived, resulting in a lysine-to-arginine substitution at amino acid 22 of the L protein, did not prevent rescue of mumps virus, even though an amino acid alignment for the L proteins of paramyxoviruses indicates that lysine is highly conserved at that position. This system may provide the basis of a safe and effective virus vector for the *in vivo* expression of immunologically and biologically active proteins, peptides, and RNAs.

Insertion of Foreign Genes

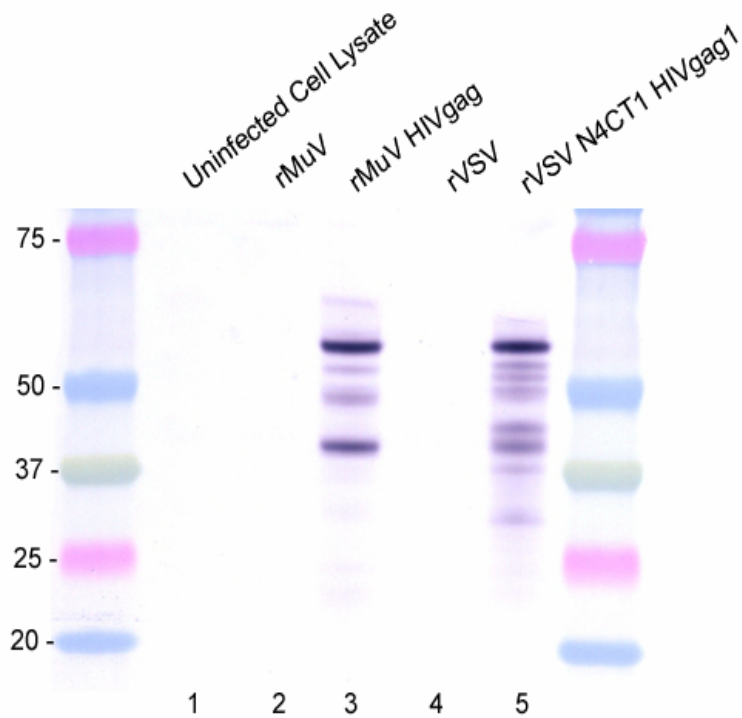


Expression of Reporter Genes (B-gal)

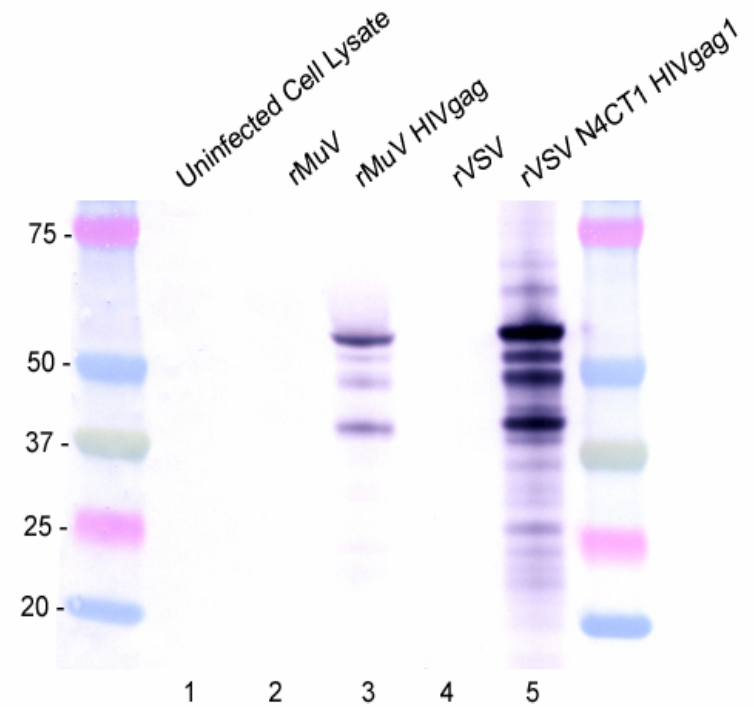


HIV-1 gag Expression

24 hrs post-infection

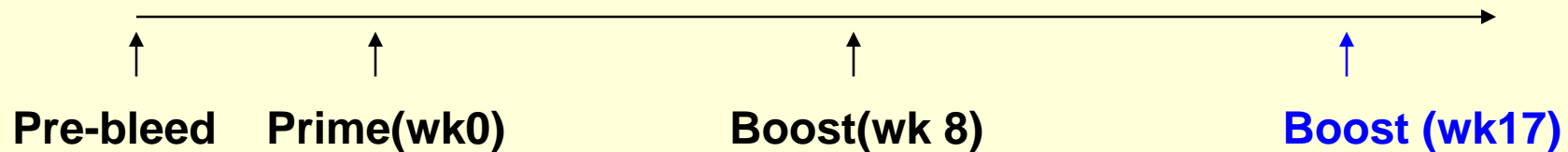


48 hrs post-infection

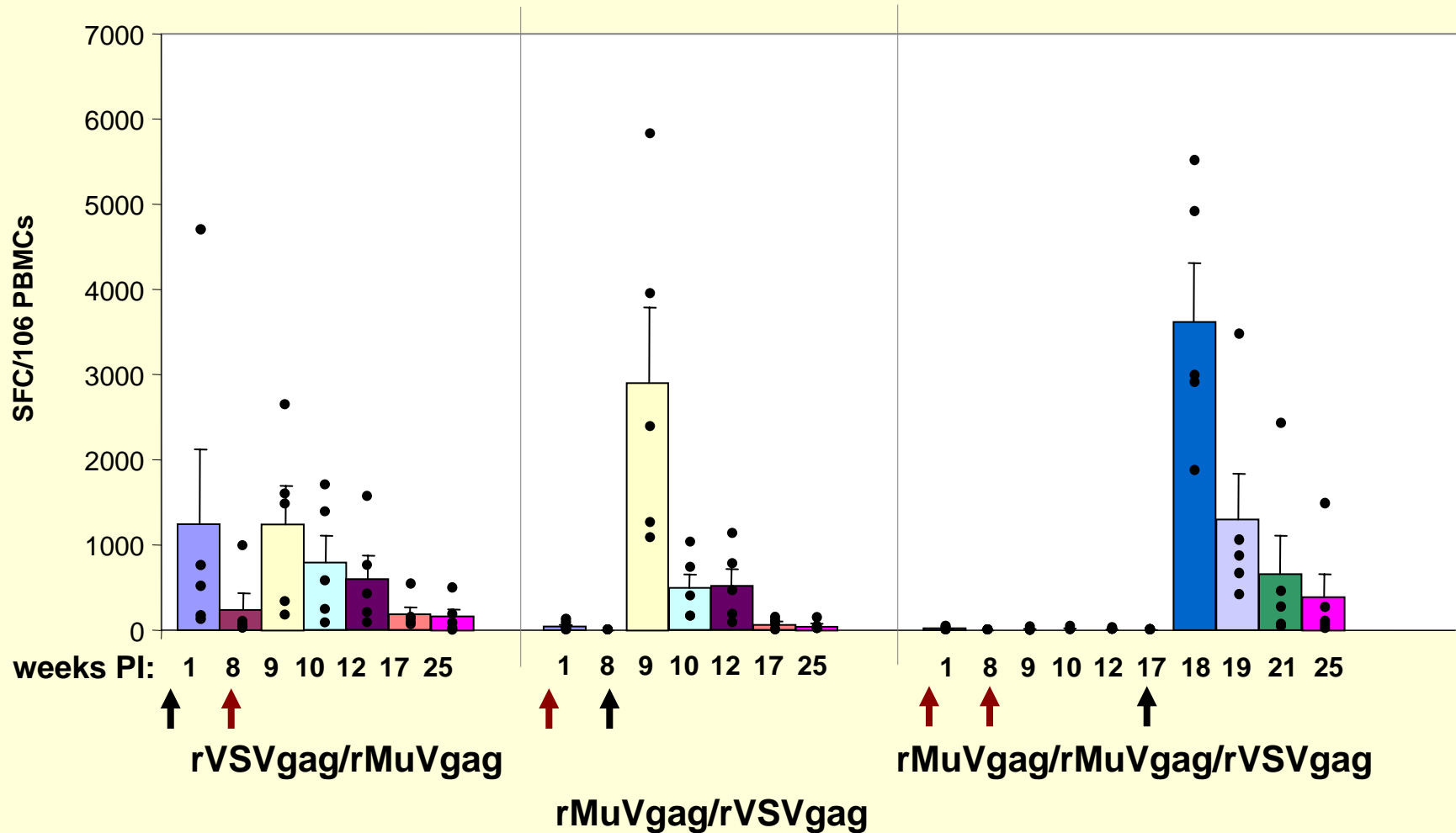


rMuVgag/rVSVgag Prime/Boost Regimens

Group	Prime	Route	Dose (pfu)	Boost	Route	Dose (pfu)	Boost	Route	Dose (pfu)	N
1	rMuV gag	SC	1×10^7	rMuV gag	SC	1×10^7	rVSV gag	IM	1×10^7	5
2	rMuV gag	SC	1×10^7	rVSV gag	IM	1×10^7				5
3	rVSV gag	IM	1×10^7	rMuV gag	SC	1×10^7				5

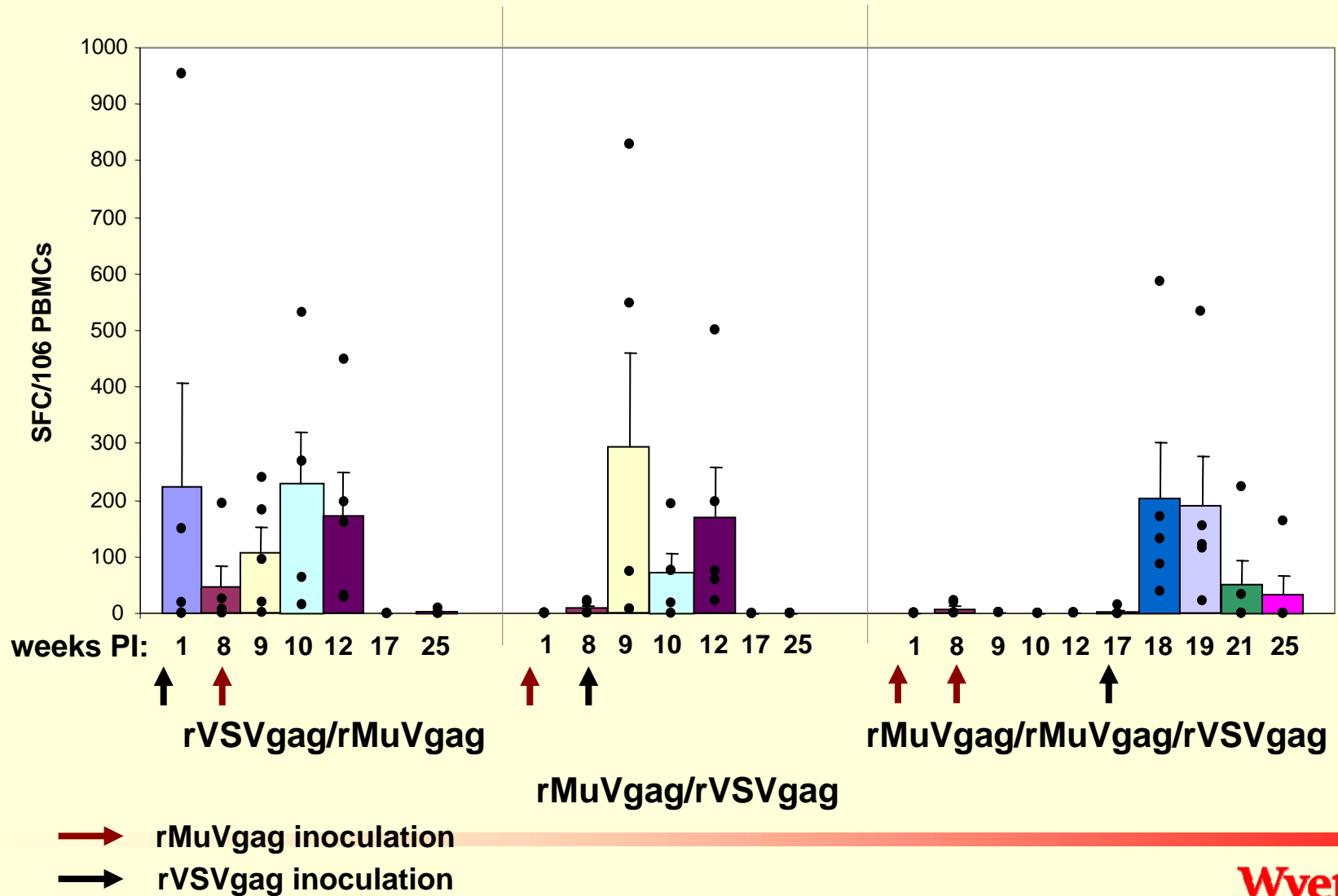


HIV-1 Gag specific IFN- γ ELISPOT responses

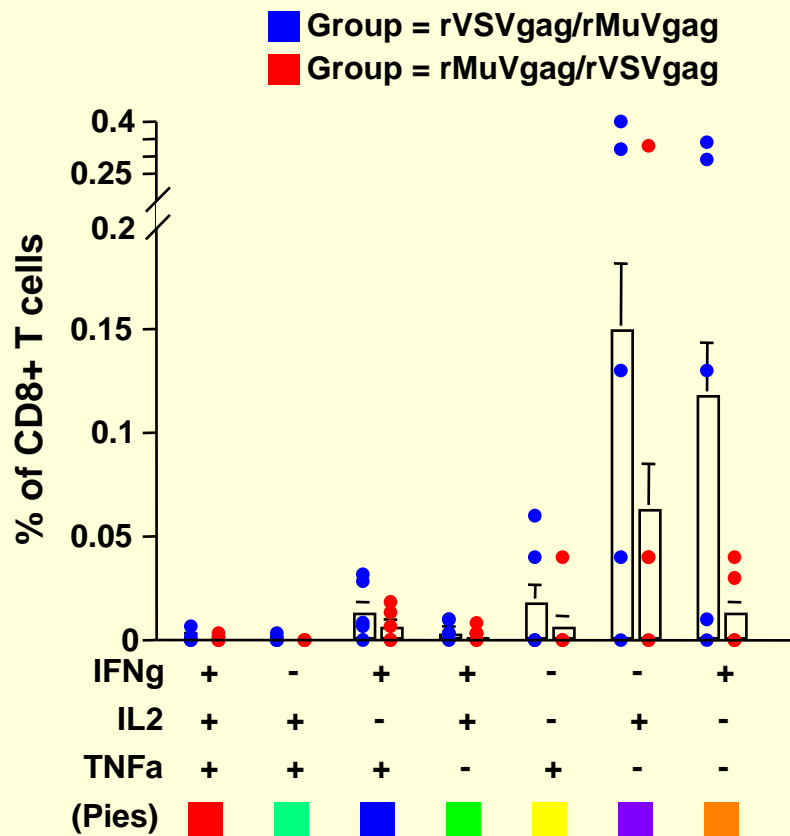


- rMuVgag inoculation
- rVSVgag inoculation

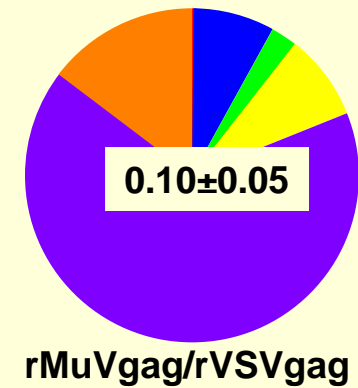
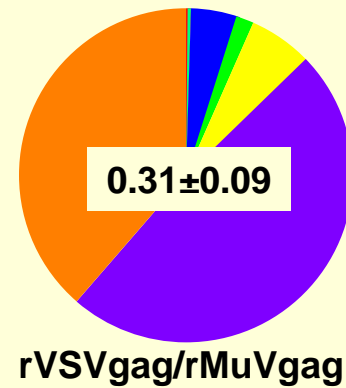
HIV-1 Gag specific IL-2 ELISPOT responses



Gag specific CD8⁺ T cell responses measured by ICS at week 25 post inoculation

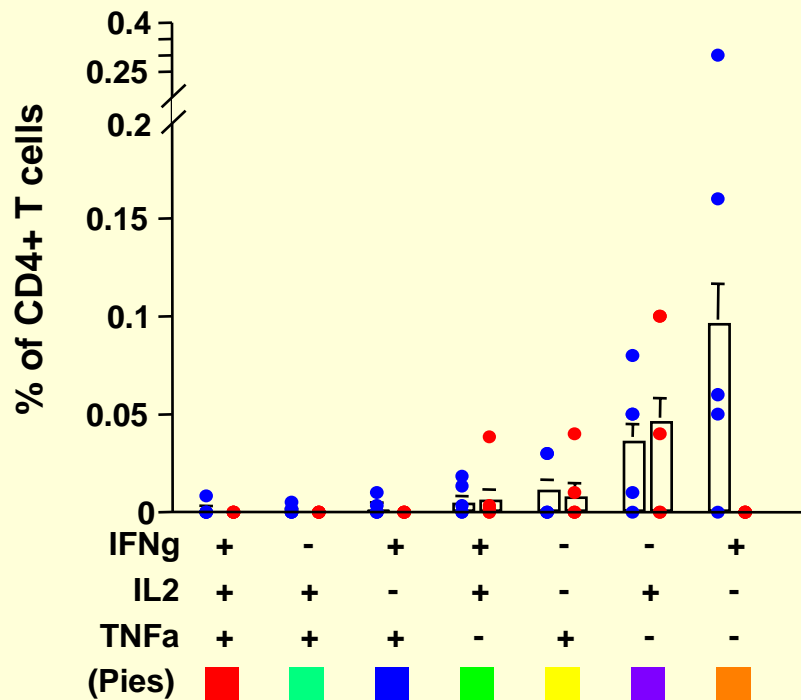


Percentage of total CD8⁺ T cells responses

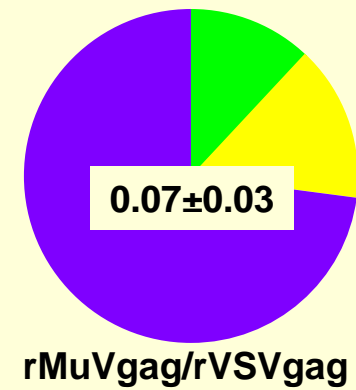
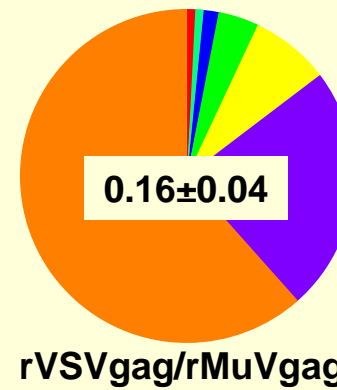


Gag specific CD4⁺ T cell responses measured by ICS at week 25 post inoculation

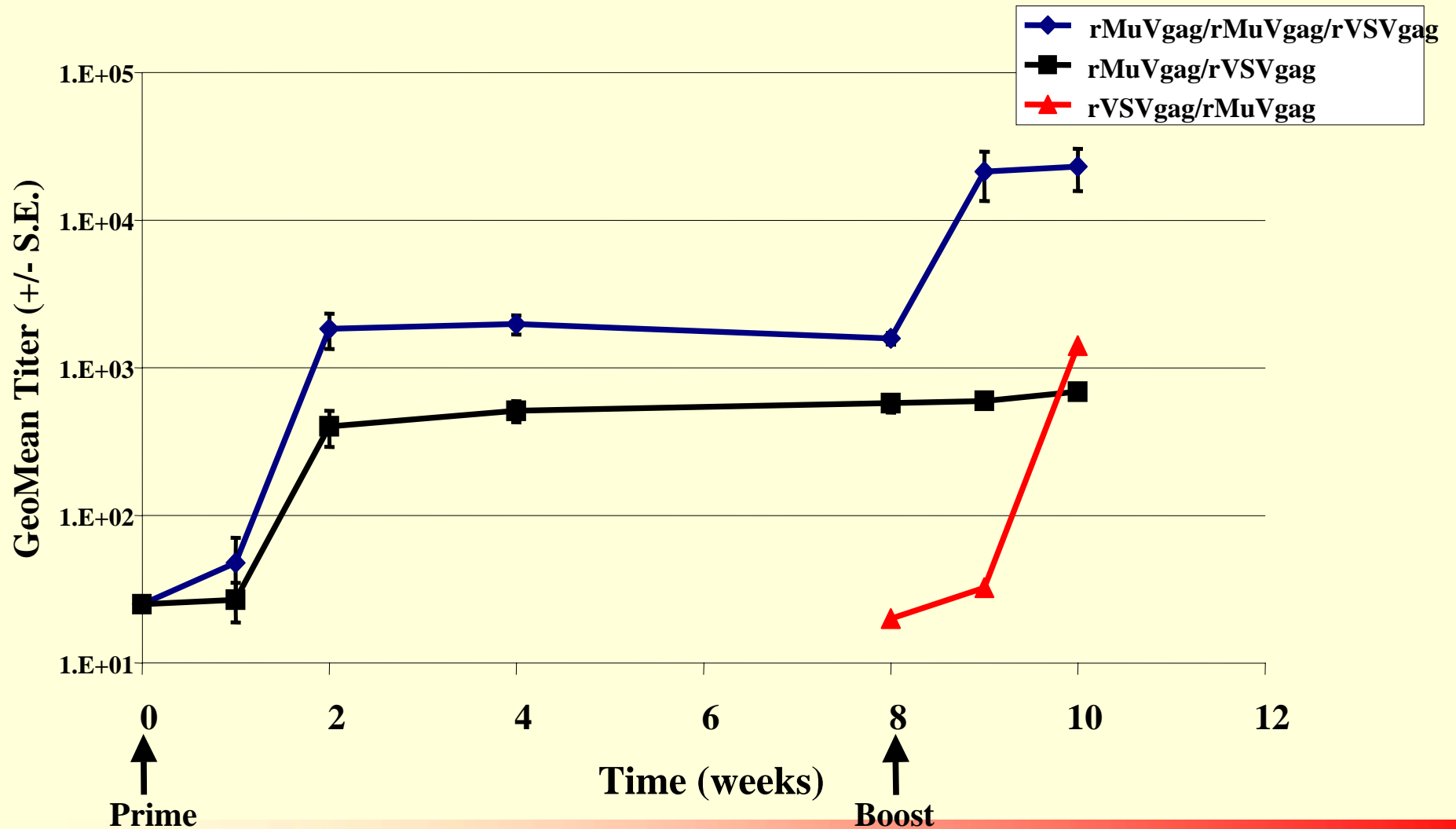
■ Group = rVSVgag/rMuVgag
■ Group = rMuVgag/rVSVgag



Percentage of total CD4⁺ T cells responses



Mumps specific IgG responses



Conclusions

- **rMuV can be modified to express HIV-1 gag**
- **Very robust gag-specific cellular immune responses were elicited in NHPs primed once or twice with rMuVgag and boosted with rVSVgag**
- **In all ten animals receiving this regimen, ≥ 1000 peak IFN- γ SFC were detected**
- **Animals primed with rVSVgag and boosted with rMuVgag also had robust gag-specific responses which may persist at higher level than in animals primed with rMuVgag and boosted with rVSVgag**

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