CMV VACCINE VECTOR-INDUCED PROTECTION AGAINST SIV IN MAURITIAN CYNOMOLGUS MACAQUES

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Abstract:

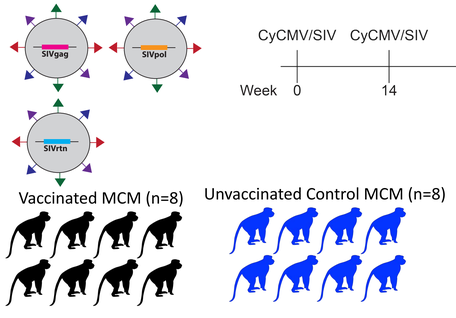
Background: Strain 68-1 rhesus cytomegalovirus (CMV) vaccine vectors expressing simian immunodeficiency virus (SIV) antigens (RhCMV/SIV) prime broadly-targeted, unconventionally MHC-II- and MHC-E-restricted CD8+ T cell responses that stringently control SIV replication in vaccinated rhesus macaques (RM). However, RM express many more MHC-II and MHC-E alleles than humans, and it remains unclear if the unprecedented cellular immunity and control of SIV observed in RhCMV/SIV-vaccinated RM is due to the unique immunogenetics of RM or species-specific functions of RhCMV itself. In contrast to RMs, Mauritian cynomolgus macaques (MCM) exhibit reduced genetic diversity with immunogenetics that more closely resemble those of humans. However, 68-1 RhCMV was unable to elicit unconventionally restricted CD8+ T cells in MCM suggesting a species barrier for viral vector function.

Methods: To determine if non-classical T cell priming and protection against mucosal SIV challenge is restricted to RhCMV-vaccination of RM or a universal phenomenon, we constructed a '68-1 like' cynomolgus CMV expressing SIV antigens (CyCMV/SIV). We vaccinated eight MCM with CyCMV/SIV and monitored multiple immune parameters in the animals including transgene-specific CD4+ and CD8+ T cell responses in blood and BAL. We challenged the eight vaccinated MCM and eight unvaccinated controls with repeated, limiting-dose, intrarectal SIVmac239 to assess vaccine-mediated protection.

Results: CyCMV/SIV vaccinated MCM generated unconventionally, MHC-II- and MHC-E-restricted T cell responses comparable to RhCMV/SIV vaccinated rhesus macaques. Upon challenge with SIVmac239, 50% of CyCMV/SIV vaccinated MCM stringently controlled SIVmac239 replication, defined as no plasma viremia and the development of T cell responses against SIV proteins absent from the vaccine. Acquisition and subsequent control of SIV was confirmed by cell-associated viral loads and adoptive transfer to naïve MCM of tissue biopsies from CyCMV/SIV-protected animals.

Conclusions: Thus, we have confirmed the distinct immunologic and protective phenotype induced by CMV vaccines in a second nonhuman primate species with immunogenetics reflective of humans, indicating that these results are not unusual species-specific traits of RM or RhCMV and that 68-1 like HCMV/HIV vaccines might similarly recapitulate unconventional T cell restriction and protect against HIV.

1 NHP Protocol: MCM vaccination with strain 68-1 CyCMV/SIV and subsequent challenge



Eight MCM were vaccinated with each of three CyCMV vectors expressing Gag, Pol, or RTN subcutaneously over three peripheral lymph nodes. Animals received a homologous boost at 14 weeks post-prime. MCM were monitored for another 45 weeks and then challenged intrarectally with limiting-dose SIVmac239 until positive viral loads were detected or Vif- and/or Env-specific CD8+ T cell responses (proteins absent from the vaccine vectors) were detected. Post-SIV acquisition, we monitored plasma viral loads, cell associated viral loads, and T cell responses. Eight concurrent unvaccinated controls were similarly challenged and monitored during the challenge and post-acquisition phase of the study.

Acknowledgements

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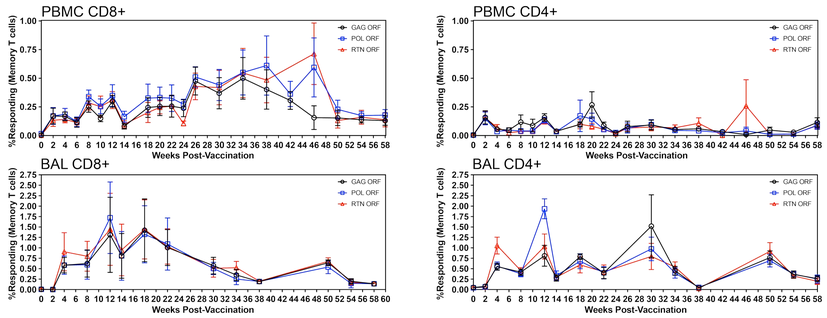
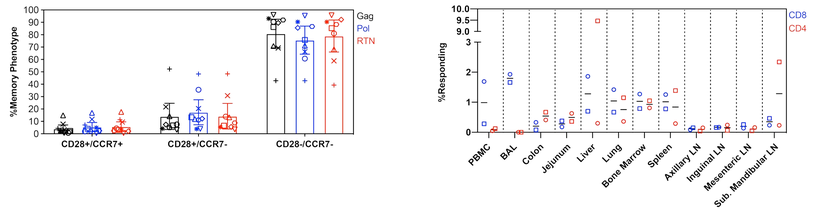
2 Longitudinal tracking of transgene-specific T cell responses



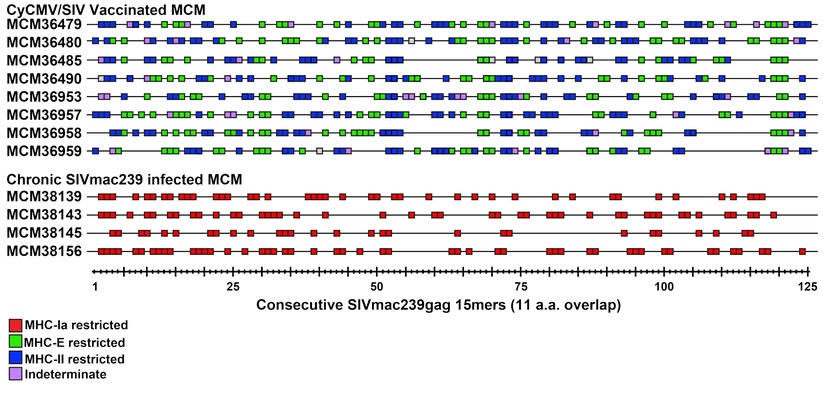
PBMC and BAL from CyCMV/SIV vaccinated macaques were incubated with ORFs for each transgene and analyzed by intracellular cytokine staining (ICCS).

3 Memory phenotype and tissue distribution of CyCMV/SIV induced T cells



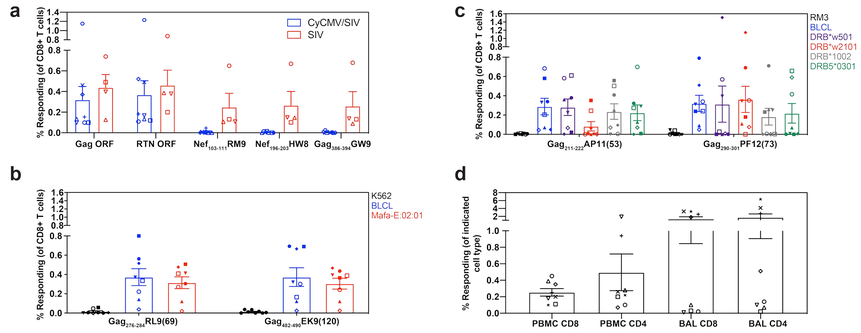
We used ICCS in combination with anti-CD28 and anti-CCR7 antibodies to identify the memory phenotype of transgene-specific CD8+ T cells in the blood (left panel). We also assessed Gag-specific CD4+ and CD8+ T cell frequencies in multiple tissues at the time of necropsy from two pilot MCM (right panel).

4 CyCMV/SIV-induced T cell specificity and MHC-restriction



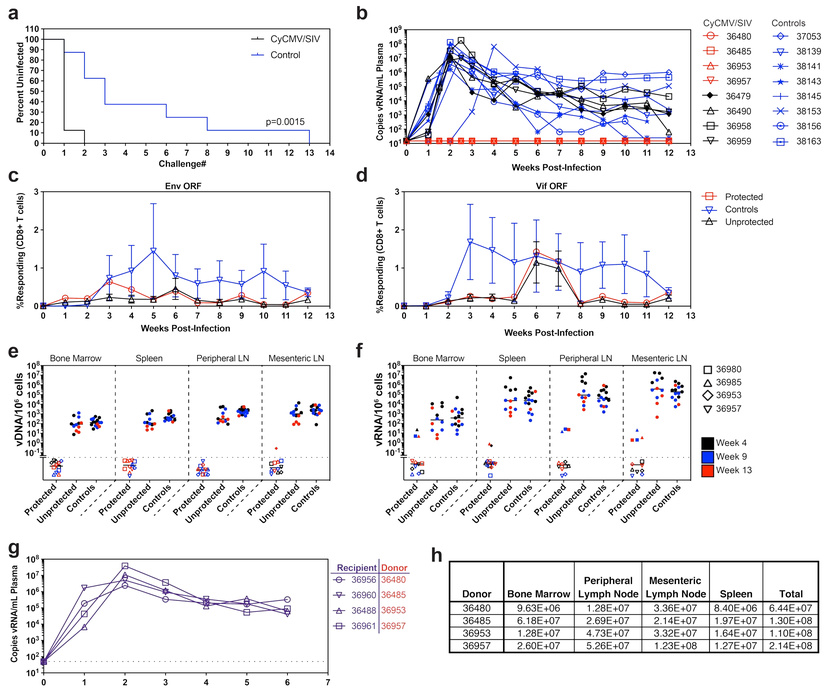
PBMCs from CyCMV/SIV (top) and SIVmac239 infected (bottom) MCM were combined with individual 15aa peptides that overlap to span the Gag ORF. Positive responses are indicated by a box. Responses were confirmed and MHC-restriction was established in an ICCS combining PBMC, peptide, and MHC blocking reagents - isotype control, anti-W6/32 (anti-MHC-I), VL9 (MHC-E blocking), or CLIP and anti-HLA-DR (MHC-II blocking). Colors indicate MHC-restriction.

5 Confirmation of unconventional T cell responses and their ability to recognize SIV-infected CD4+ T cells



a) CyCMV/SIV induced CD8+ T cells do not recognize canonical MHC-Ia restricted peptides. b) MHC-E and c) MHC-II restricted CD8+ T cells recognize supertopes on single allomorph expressing (b) K562 and (c) RM3 cell lines. d) CD4 and CD8+ T cells from CyCMV/SIV vaccinated PBMC and BAL recognize SIV-infected target cells.

6 Intrarectal, limiting dose, SIVmac239 challenge of eight vaccinated and eight unvaccinated control macaques



Donor	Bone Marrow	Peripheral Lymph Node	Mesenteric Lymph Node	Spleen	Total
36480	9.63E+06	1.28E+07	3.36E+07	8.40E+06	6.44E+07
36485	6.18E+07	2.69E+07	2.14E+07	1.97E+07	1.30E+08
36953	1.28E+07	4.73E+07	3.32E+07	1.64E+07	1.10E+08
36957	2.60E+07	5.26E+07	1.23E+08	1.27E+07	2.14E+08

a) Percent of MCM remaining uninfected during repeated limiting-dose SIVmac239, intrarectal challenge of unvaccinated controls and CyCMV/SIV vaccinated animals. b) Post-infection measurement of vRNA/mL plasma. c) Measuring Env- and d) Vif-specific T cell responses in MCM post-acquisition. e) Cell associated vDNA and f) vRNA measurements from MCM at several time-points and tissues post acquisition. g) Adoptive transfer of tissues indicated in (h) from CyCMV/SIV vaccinated, protected MCM into naïve recipients to demonstrate that donor animals were infected despite undetectable plasma viral loads. h) Number of cells transferred from each donor to each recipient.

Conclusions

1. CyCMV/SIV vaccination of MCM induces unconventional MHC-II and MHC-E restricted CD8+ T cells and 'supertope' specific responses that are identical in specificity to those induced by RhCMV/SIV vaccination of rhesus macaques.
2. Fifty percent of CyCMV/SIV vaccinated MCM stringently control SIV infection.
3. The frequency of acquisition may be increased in CyCMV/SIV vaccinated MCM