

Modulation of DNA Vaccine-Elicited CD8+ T Lymphocyte Epitope Immunodominance Hierarchies

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Immunodominance of Epitope-Specific CD8+ T Lymphocyte Responses

- **CD8+ T lymphocyte responses during a typical infection are highly focused with narrow breadth**
- **Of all possible peptides that bind MHC class I, few are actual CD8+ T lymphocyte epitopes**
- **Determinants of immunodominance:**
 - Adequate antigen expression
 - Efficient antigen processing
 - Competition among peptides for MHC
 - MHC/peptide binding affinity
 - T lymphocyte repertoire
 - ?

Rationale for Expanding Breadth of CD8+ T Lymphocyte Responses for an HIV Vaccine

- **Goal: To increase subdominant responses and improve breadth of cellular immune responses**
 - **May increase vaccine coverage given viral diversity**
 - **May reduce frequency and consequences of viral escape from CD8 T lymphocytes**
 - **May improve protective efficacy and durability of protection**
- **However, immunodominance hierarchies are often fixed and typically difficult to change**

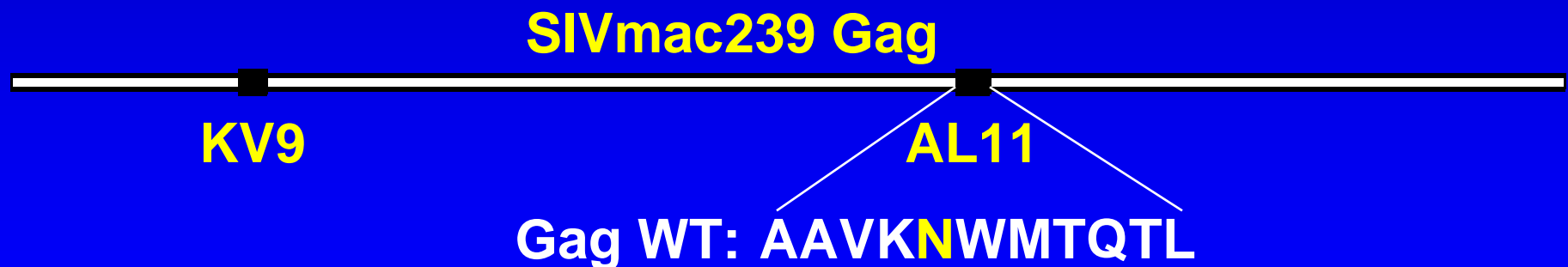
Experimental System: D^b-restricted CD8⁺ T Lymphocyte Responses to SIVmac239 Gag in C57/BL6 Mice

- D^b-restricted CD8⁺ T lymphocyte epitopes in SIVmac239 Gag:
 - Dominant AL11 epitope: **AAVKNWMTQTL**
 - Subdominant KV9 epitope: **KSLYNTVCV**
- Aims:
 - To assess the degree that KV9 subdominance to AL11 is due to “immunodomination”
 - To develop novel HIV vaccine strategies that selectively increase responses to subdominant epitopes



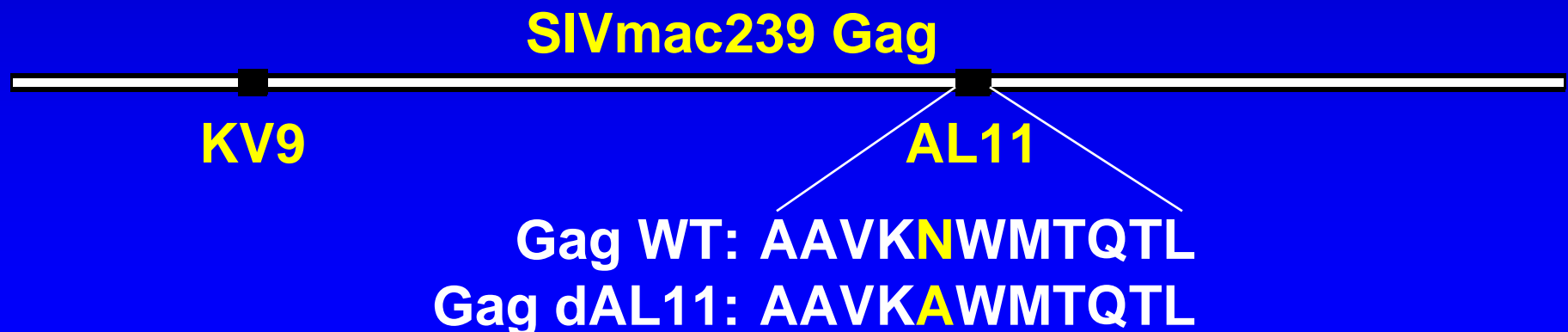
Deletion of Immunodominant AL11 Epitope in SIVmac239 Gag

- Experimental strategy:
 - Delete the immunodominant AL11 epitope in SIV Gag
 - Assess responses to KV9 and AL11 in mice immunized with DNA vaccines expressing Gag WT or Gag dAL11



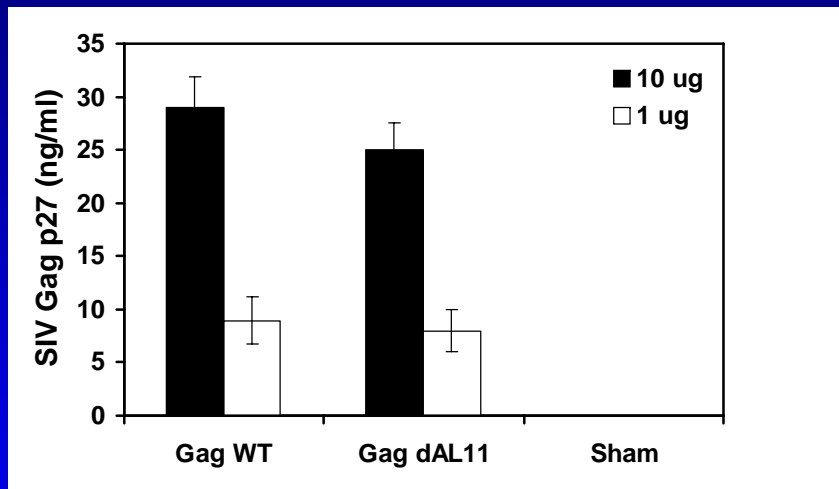
Deletion of Immunodominant AL11 Epitope in SIVmac239 Gag

- Strategy to delete AL11:
 - Incorporate point mutation to change the position 5 anchor residue of AL11 (N) to abrogate binding to D^b
 - Gag dAL11 (N->A)



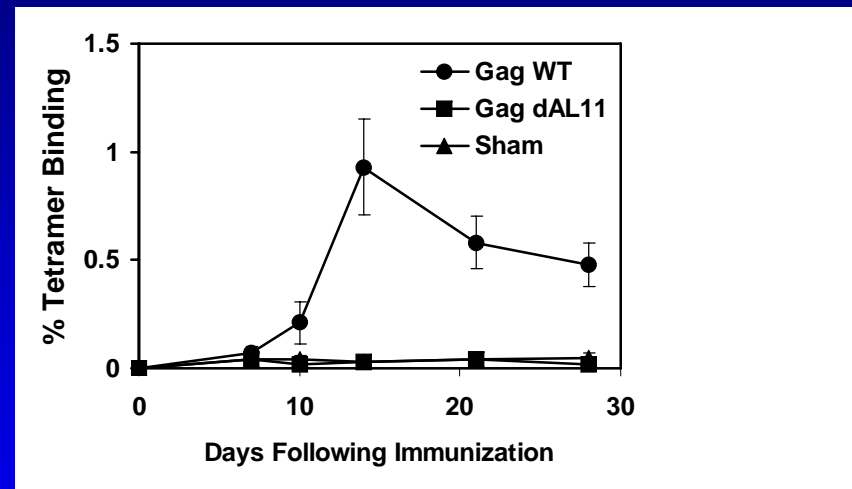
Preliminary Evaluation of the Plasmid DNA Vaccine Expressing SIV Gag dAL11

Gag p27 ELISA



- Transiently transfected 10 ug or 1 ug DNA in vitro
- Gag p27 was detected by mAbs in an ELISA
- **Gag WT and Gag dAL11 plasmids express similar levels of Gag protein**

AL11 Tetramer Binding



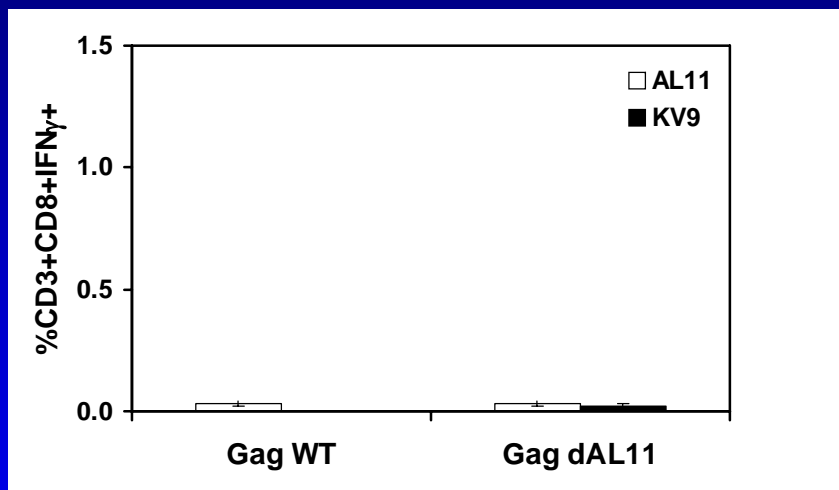
- Injected C57/BL6 mice with 50 ug DNA i.m.
- AL11-specific responses were detected by D^b/AL11 tetramer binding assays
- **Gag dAL11 plasmid elicits no AL11-specific responses**

AL11- and KV9-Specific CD8+ T Lymphocyte Responses Elicited by DNA Vaccines Expressing Gag WT and Gag dAL11

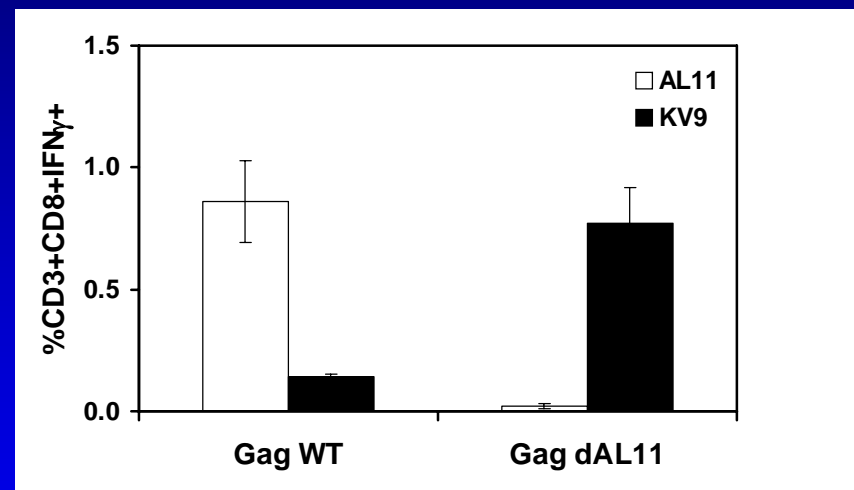
- **C57/BL6 mice (N=12/group) injected at weeks 0 and 4 i.m. with:**
 - 50 ug DNA expressing Gag WT
 - 50 ug DNA expressing Gag dAL11
- **AL11- and KV9-specific CD8+ T lymphocyte responses were assessed by:**
 - IFN- γ ICS assays using PBMC
 - IFN- γ ELISPOT assays using splenocytes

AL11- and KV9-Specific CD8+ T Lymphocyte Responses Elicited by DNA Vaccines Expressing Gag WT and Gag dAL11

Week 0 (ICS)

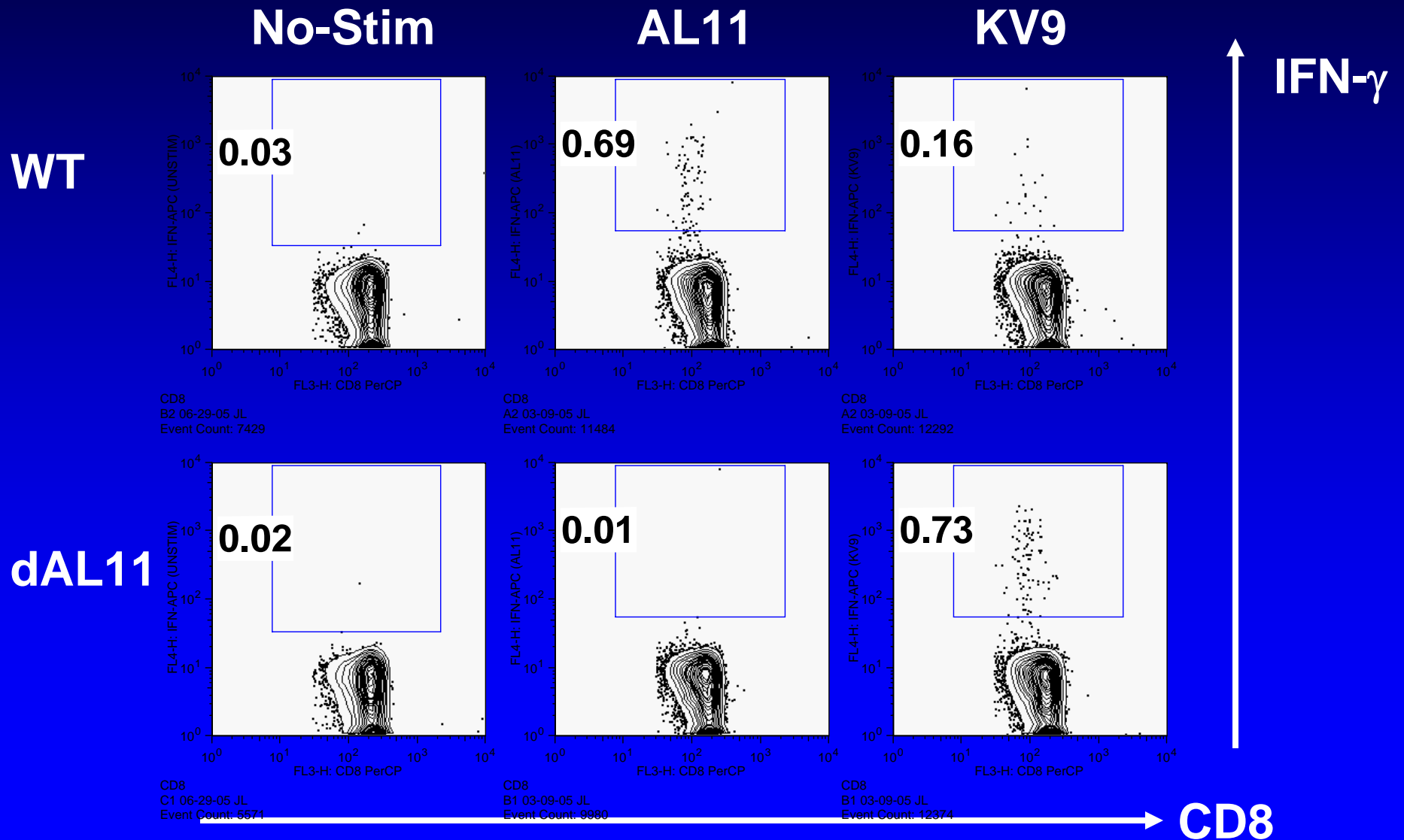


Week 2 (ICS)



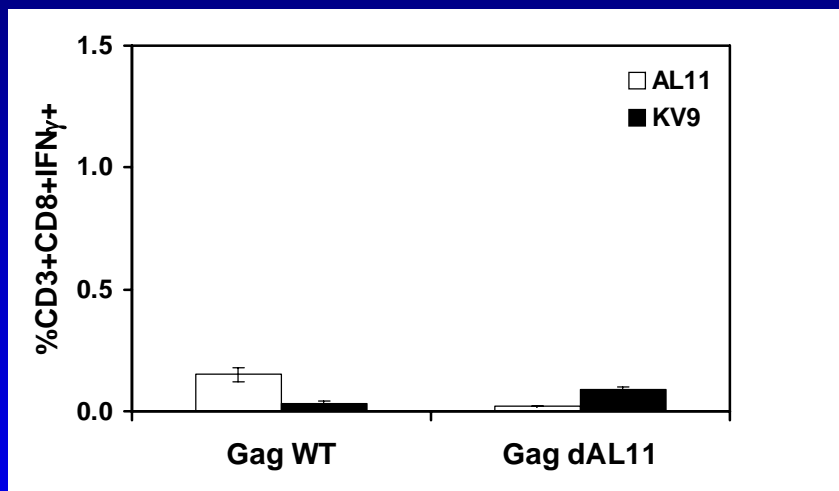
- Gag WT: dominant AL11, subdominant KV9 responses
- Gag dAL11: no AL11 responses, enhanced KV9 responses
- KV9 responses elicited by Gag dAL11 were comparable in magnitude to AL11 responses elicited by Gag WT
- Thus, KV9 responses were enhanced by deletion of AL11, suggesting that in Gag WT, AL11 “immunodominates” KV9

Intracellular Cytokine Staining (ICS) Assays Using PBMC From Mice

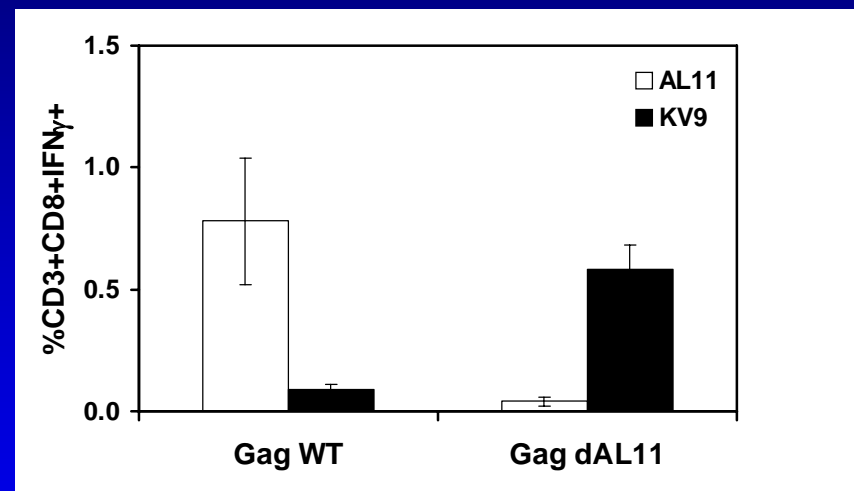


AL11- and KV9-Specific CD8+ T Lymphocyte Responses Elicited by DNA Vaccines Expressing Gag WT and Gag dAL11

Week 4 (ICS)



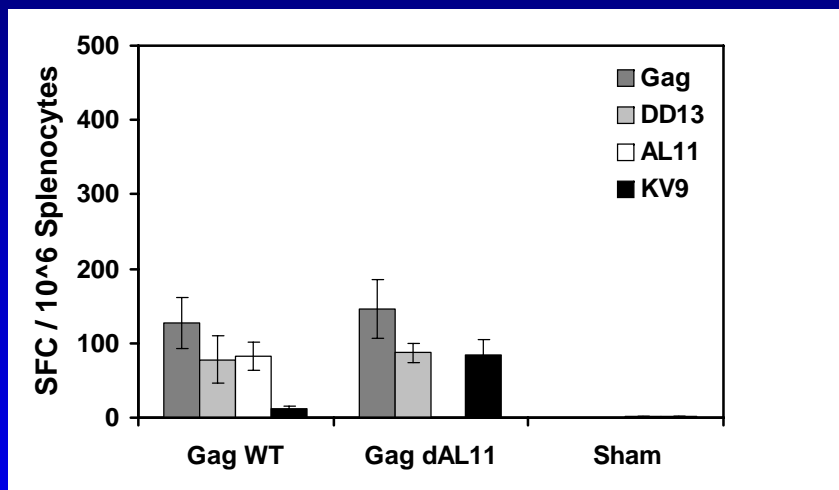
Week 7 (ICS)



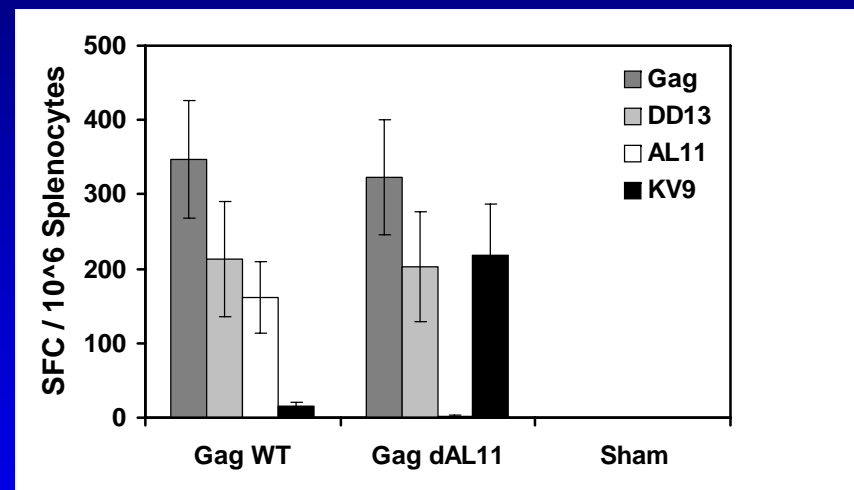
- At week 4, responses diminished in magnitude
- Following the boost immunization, the relative KV9 and AL11 hierarchies elicited by Gag WT and Gag dAL11 persisted

AL11- and KV9-Specific CD8+ T Lymphocyte Responses Elicited by DNA Vaccines Expressing Gag WT and Gag dAL11

Week 4 (ELISPOT)



Week 8 (ELISPOT)



- Deletion of D^b-restricted AL11 epitope results in:
 - No overall change in total Gag peptide pool responses
 - No change in MHC class II-restricted DD13 responses
 - Complete ablation of D^b-restricted AL11 responses
 - Marked enhancement of D^b-restricted KV9 responses

Immunogenicity of Prime-Boost DNA-rAd5 Vaccine Regimens Containing Gag WT and Gag dAL11

- **In a practical DNA-rAd5 prime-boost regimen, can we expand vaccine breadth?**
 - Can we enhance subdominant responses without loss of the dominant responses?
- **rAd5 is intrinsically more potent than DNA, and thus dominant epitopes may not need DNA priming**
- **A potential vaccine strategy is therefore:**
 - To prime with DNA expressing Gag dAL11 to prime potent responses to KV9
 - To boost with rAd5 expressing Gag WT to expand existing KV9 responses and prime new AL11 responses

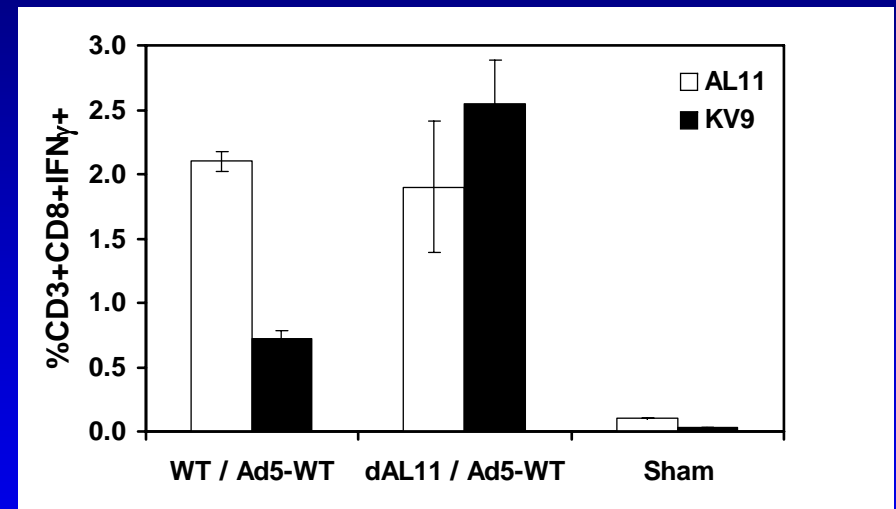
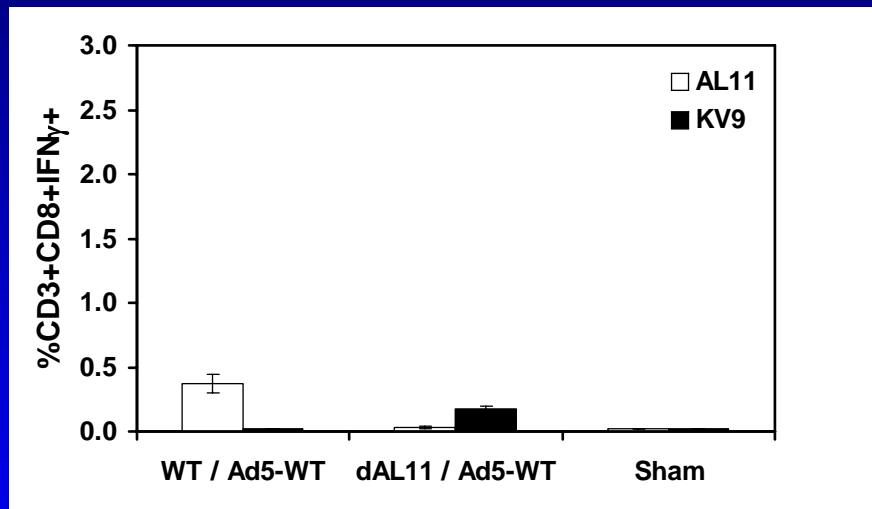
Immunogenicity of Prime-Boost DNA-rAd5 Vaccine Regimens Containing Gag WT and Gag dAL11

- C57/BL6 mice (N=8/group) primed at week 0 and boosted at week 4 i.m. with:
 - 50 μ g DNA-Gag WT / 10^6 vp Ad5-Gag WT
 - 50 μ g DNA-Gag dAL11 / 10^6 vp Ad5-Gag WT
 - 50 μ g DNA-Sham / 10^6 vp Ad5-Sham
- Mice were challenged at week 10 with 5×10^6 pfu vaccinia-Gag
- Assays included:
 - IFN- γ ICS assays using PBMC
 - IFN- γ ELISPOT assays using splenocytes
 - Vaccinia pfu assays using ovaries following challenge

Immunogenicity of DNA/rAd5 Prime-Boost Vaccine Regimens Containing Gag WT and Gag dAL11 (After Week 4 rAd5 Boost)

Week 4 (ICS)

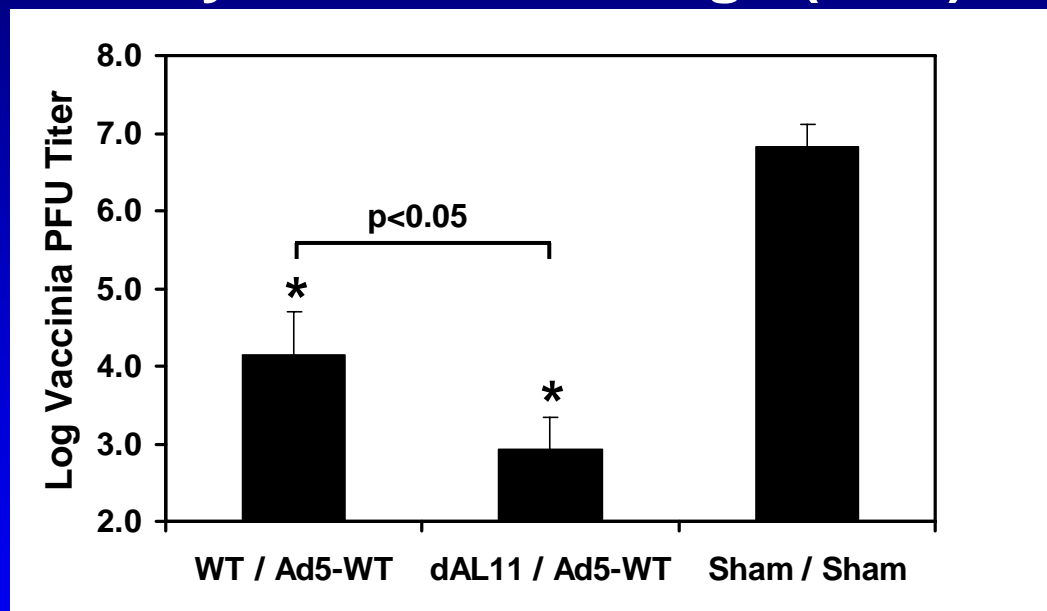
Week 6 (ICS)



- Deletion of AL11 from the DNA prime resulted in increased KV9 responses but preserved AL11 responses following the Ad5 boost
- Therefore, the DNA-Gag-dAL11/Ad5-Gag-WT regimen resulted in balanced, co-dominant AL11 and KV9 responses

Protective Efficacy of DNA/rAd5 Vaccine Regimens Against Vaccinia-Gag Challenge

Day 6 After Challenge (PFU)



- Both DNA/rAd5 regimens afforded significant 2-4 log reductions of Vac-Gag titers ($p < 0.01$)
- Priming with DNA-Gag-dAL11 was more effective than priming with DNA-Gag-WT in protecting against Vac-Gag ($p < 0.05$)

Conclusions

- Deletion of the dominant D^b-restricted AL11 epitope in SIV Gag DNA vaccine results in:
 - Markedly enhanced responses to the subdominant D^b-restricted KV9 epitope in mice (immunodomination)
 - Did not affect CD4 responses to DD13
- These data suggest that immunodomination is specific to the relevant MHC class I restricting allele
- Heterologous prime-boost regimens can be designed to elicit balanced, co-dominant AL11 and KV9 responses

Conclusions

- **DNA priming without the dominant epitope followed by rAd5 boosting with the wildtype antigen resulted in:**
 - **Enhanced subdominant responses**
 - **Minimal loss of dominant responses**
 - **Improved protective efficacy to vaccinia challenge in mice**
- **Epitope modification could potentially be beneficial in HIV vaccines to broaden CD8+ T lymphocyte responses:**
 - **May increase vaccine coverage given viral diversity**
 - **May minimize viral escape from CD8+ T lymphocytes**
- **Studies in rhesus monkeys are currently in progress**

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