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: \( \text{D}^b \)-restricted CD8+ T Lymphocyte Responses to SIVmac239 Gag in C57/BL6 Mice

- \( \text{D}^b \)-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

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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<sup>b</sup>/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

**WT**

<table>
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<th>Condition</th>
<th>CD8</th>
<th>IFN-γ</th>
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<tr>
<td>AL11</td>
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<td>KV9</td>
<td>0.16</td>
<td>0.73</td>
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**dAL11**

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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 μg DNA-Gag WT / 10^6 vp Ad5-Gag WT
  – 50 μg DNA-Gag dAL11 / 10^6 vp Ad5-Gag WT
  – 50 μg DNA-Sham / 10^6 vp Ad5-Sham

• Mice were challenged at week 10 with 5 x 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)

- 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.
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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