

**Abrogation of *in vivo* Efficacy of Vaccine-induced CTL
against Heterologous SIV Challenge by a Single Amino
Acid Change in Viral Epitope Flanking Region**

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ABSTRACT

A current promising strategy for AIDS vaccine development is to elicit virus-specific cytotoxic T lymphocyte (CTL) responses that broadly recognize highly-diversified HIVs. However, it has remained unclear how broadly vaccine-induced CTLs can recognize heterologous viruses *in vivo*. In our previous preclinical vaccine trial eliciting simian immunodeficiency virus (SIV) mac239 Gag-specific CTL responses, we have found a group of Burmese rhesus macaques possessing an MHC class I haplotype *90-120-Ia* which showed vaccine-based viral control against a homologous SIV_{mac239} challenge (J Exp Med 199:1709, 2004). Vaccine-induced Gag₂₀₆₋₂₁₆ epitope-specific CTL responses exerted a strong selective pressure on the virus in this control.

Here, we have challenged two *90-120-Ia*-positive vaccinees with a heterologous SIV_{smE543-3} that has the same Gag206-216 epitope sequence, and unexpectedly, have found that vaccine-induced Gag206-216-specific CTLs failed to recognize this Gag206-216 epitope in the context of SIV_{smE543-3} Gag.

Despite efficient elicitation of Gag-specific CTL responses by prophylactic vaccination with a Sendai virus vector expressing SIV_{mac239} Gag, both vaccinees failed to control heterologous SIV_{smE543-3} replication (**Fig 1-2**). In the *90-120-Ia*-positive unvaccinated controls or vaccinees, the SIV_{smE543-3} challenge did not result in induction or expansion of Gag206-216-specific CTL responses nor selection of mutations within the Gag206-216 epitope region (**Fig 3**), although a Gag206-216-specific CTL escape mutation was selected in all the *90-120-Ia*-positive animals infected with SIV_{mac239}.

Further analysis indicated failure in recognition of SIV_{smE543-3}-infected cells by Gag206-216-specific CTLs due to a change from aspartate (D) in SIV_{mac239} to glutamate (E) in SIV_{smE543-3} at Gag residue 205 immediately preceding the amino terminus of Gag206-216 epitope (Fig 4-5). Although impairment of CTL epitope processing and presentation by a change in viral epitope flanking sequences has been reported in HIV-1 infections, our results show that **even vaccine-induced CTL efficacy *in vivo* can be abrogated by a single amino acid change in viral epitope flanking sequences.** This underlines the influence of viral epitope flanking sequences on CTL-based AIDS vaccine efficacy, suggesting an important implication for development of an effective vaccine against highly-diversified HIVs.

Homologous/Heterologous SIV challenge experiments in DNA/SeV-"mac239"Gag vaccinated macaques possessing MHC-I haplotype 90-120-Ia

In the previous study: Homologous SIV_{mac239} challenge

Naive	persistent viremia
Vaccinee	undetectable setpoint viremia

Gag₂₀₆₋₂₁₆-specific CTLs exerted a strong selective pressure
on SIV_{mac239} in this control

SIV _{mac239} Gag 206-216	IINEEAADWDL
SIV _{smE543} Gag 206-216	-----

In this study: Heterologous SIV_{smE543-3} challenge

Naive	R01-002, R01-003	?
Vaccinee	R00-018, R01-006	

Fig 1

Heterologous SIV_{smE543-3} challenge / MHC 90-120-Ia(+) vaccinees

Failure in viral control despite efficient Gag-specific CTL induction by vaccination

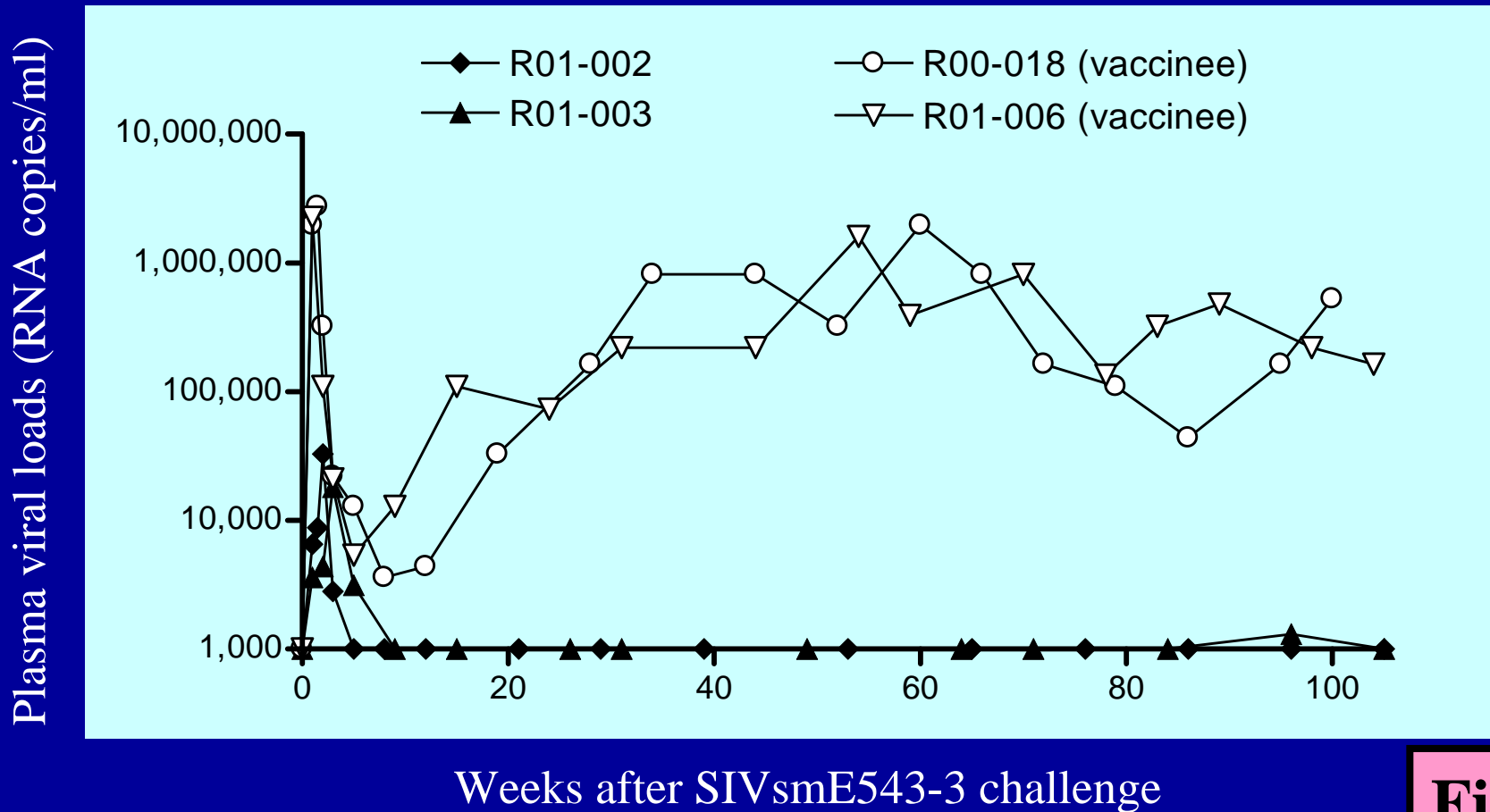
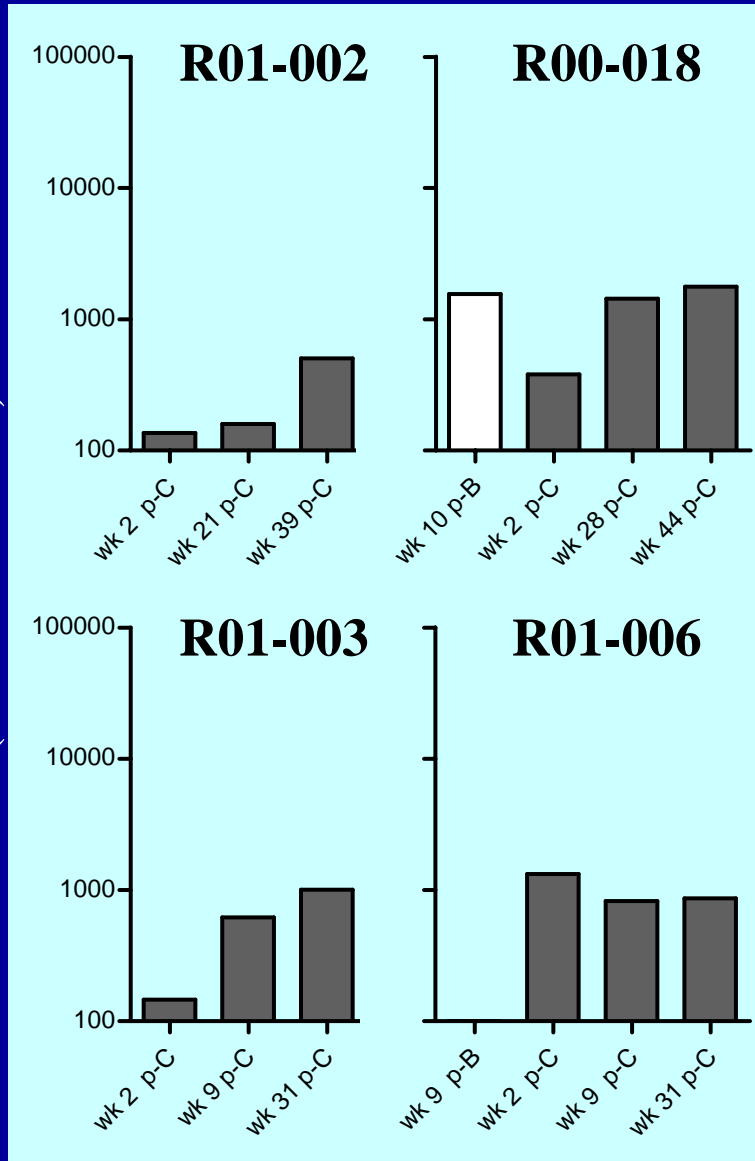


Fig 1

Vaccine-induced SIV_{mac239}-specific CTLs did not efficiently respond to SIV_{smE543-3} challenge.

SIV_{smE543-3}-specific CD8 T cell frequencies (/million PBMC)



SIV_{mac239}-specific CD8 T cell frequencies (/million PBMC)

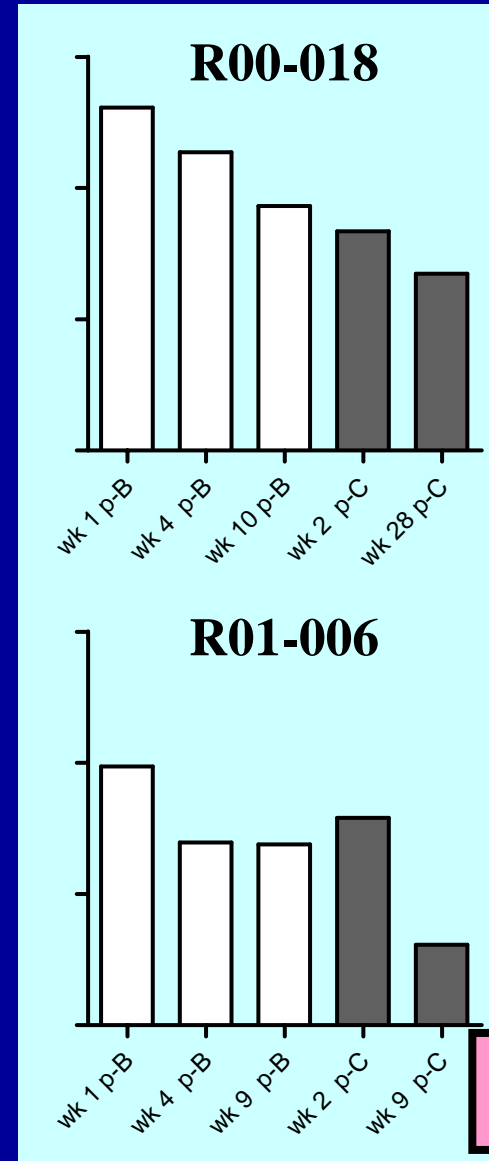


Fig 2

Gag206-216-specific CTLs did not respond to nor exert selective pressure on SIV_{smE543-3}.

No Gag206-216 CTL escape mutation

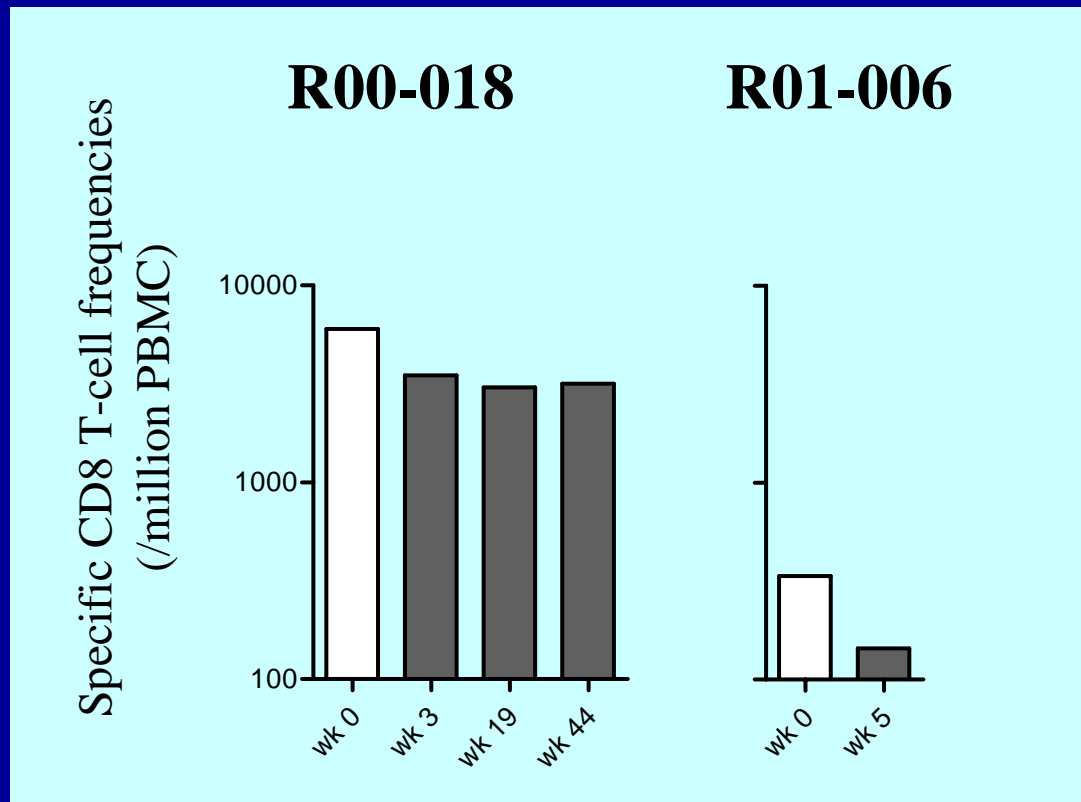


Fig 3

Gag206-216-specific CTLs recognized both Gag202-216 & Gag202-216.205E peptide-pulsed BLCLs.

Gag202-216 sequence

SIV_{mac239}

IIRD IINEEAADWDL

SIV_{smE543-3}

IIRE IINEEAADWDL

Specific CD8 T cell frequencies (/million PBMC)

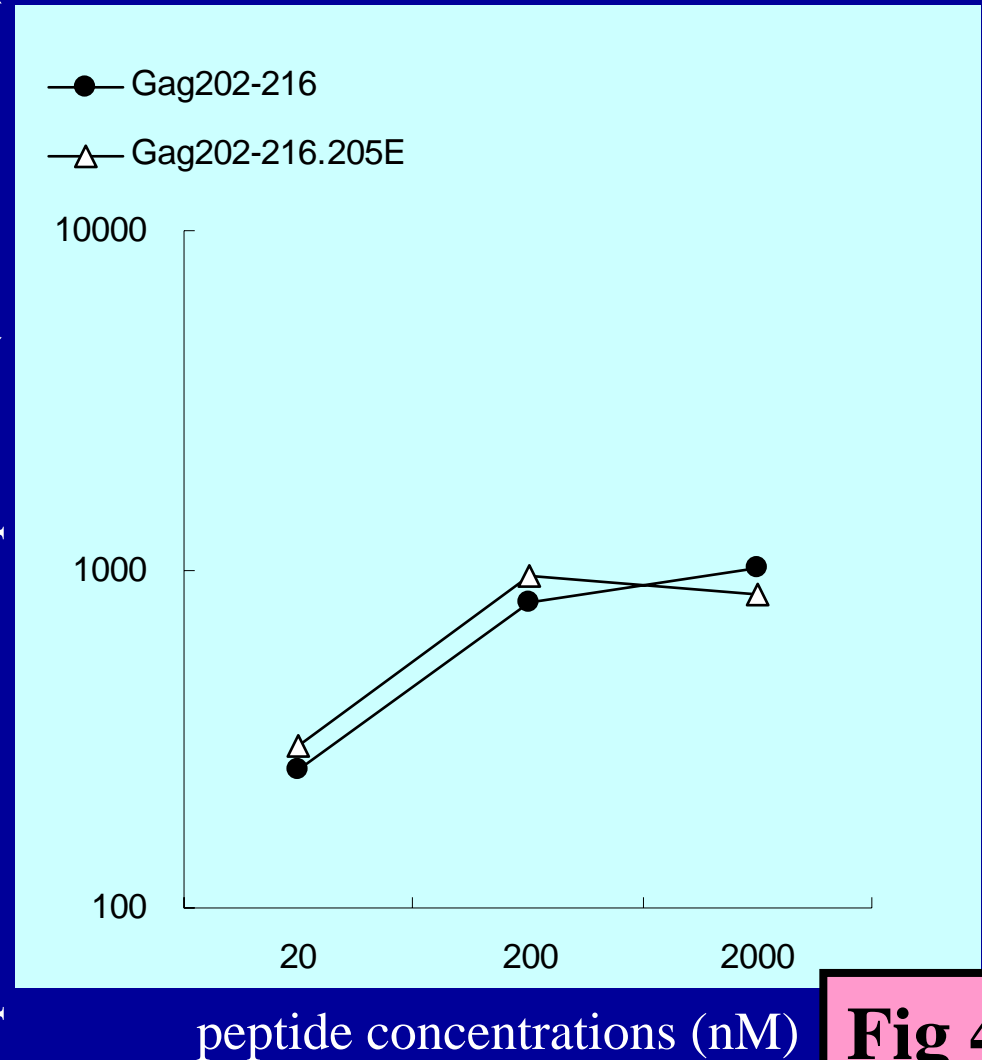


Fig 4

Failure in recognition of Gag206-216 epitope by Gag206-216-specific CTLs due to viral GagD205E change

Gag206-216-specific CTLs recognized pEGFP-N1-Gag202-216-transfected BLCL but "not" pEGFP-N1-Gag202-216.205E-transfected BLCL.

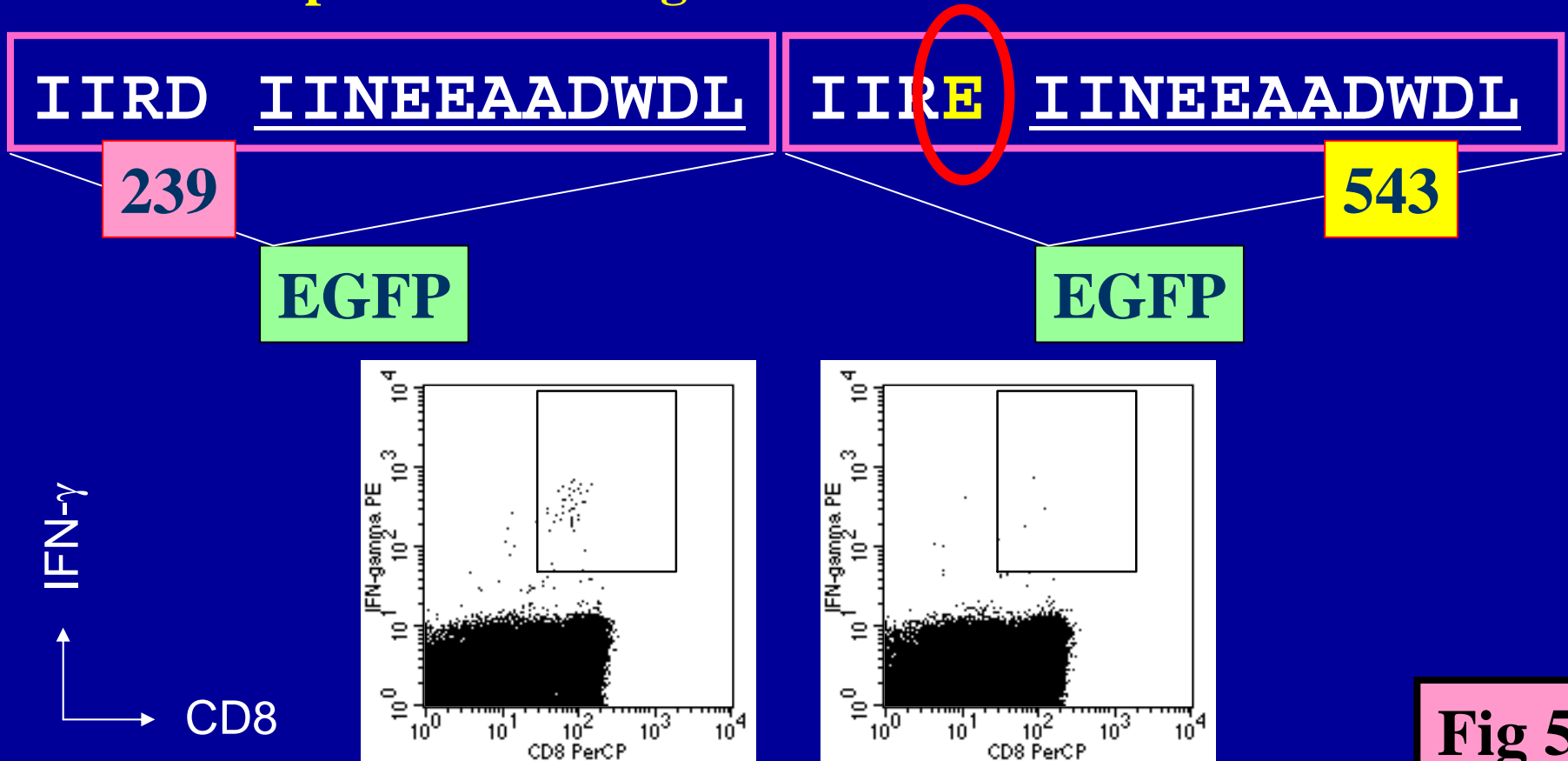


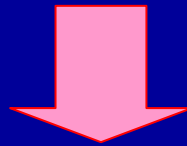
Fig 5

SUMMARY

Heterologous SIV_{smE543-3} challenge

Vaccinees with MHC 90-120-Ia

Vaccine-induced Gag206-216-specific CTL responses that exerted strong suppressive pressure on SIV_{mac239} replication were not effective against SIV_{smE543-3} challenge due to GagD205E change in viral epitope flanking region.



Even "vaccine-induced" CTL efficacy in vivo can be abrogated due to a single amino acid change in viral epitope flanking region.

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