

Figure 1. Components of V domain generation and structure, as exemplified by the BCR H chain.

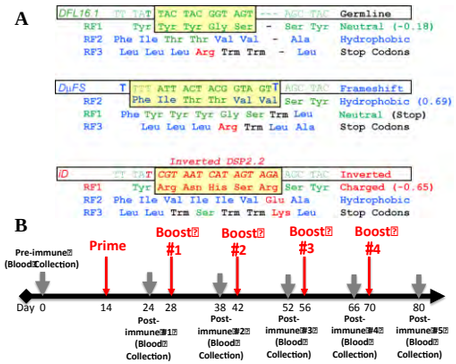


Figure 2. (A) BALB/c WT D_H and D_H altered loci. (B) Immunizations strategy.

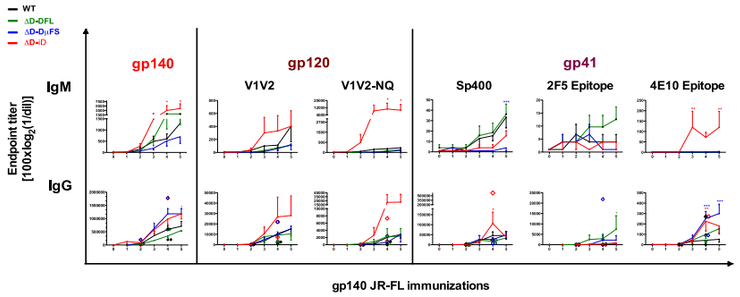


Figure 3. Alteration of D_H sequence biases anti-HIV-1 gp140 epitope preference. Specific-IgM and IgG binding to HIV-1-gp140 and neutralization-associated epitopes was assessed in WT (black) and the three strains of D-altered mice ΔD-DFL (green), ΔD-DμFS (blue), and ΔD-iD (red) sera. Individual mean endpoint titers after 1-5 JRFL gp140 i.m. immunizations are shown. The diamond symbols indicate the individual endpoint titers in the two mice selected for positive linear epitope identification by PEPperPRINT® chip. The mean and standard error of the mean are shown for responder mice. Statistical significance is determined by a *p* value <0.005 (***), <0.01 (**), <0.05(*), respectively, ANOVA.

HIV-1 Envelope Epitope Recognition is Influenced by Immunoglobulin D_H Gene Segment Repertoire

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Background

We tested whether restrictions imposed by natural selection of D_H sequence influence HIV-1 epitope recognition by immunizing wild type and D_H altered mice with HIV-1 JR-FL gp140. After immunization we used ELISA to examine serum antibody reactivity to key epitopes and overlapping peptides from HIV-1 envelope protein. Altered patterns of epitope recognition in the D_H altered mice may help explain why HIV patients may need extensive somatic mutation of their antibodies to bind to specific HIV epitopes and achieve neutralization.

Materials and Methods

Mice. We previously altered the D_H locus in BALB/c mice by deleting all but one D_H and forcing use of an alternative reading frame in that D_H (Figure 2A). DFL 16.1 mice are limited to a single WT D_H that preferentially contributes neutral amino acids to CDR-H, while ΔD-DμFS and ΔD-iD preferentially contribute RF2 encoded hydrophobic and iRF1 charged aa, respectively.

Immunization. For each strain, including WT, 5 mice were immunized intramuscularly every two weeks with HIV-1 JR-FL gp140, for a total of one primary and four booster immunizations. Blood samples were obtained 10 days after each immunization (Figure 2B).

Serum Assay. Binding ability was examined by HIV-1 envelope protein ELISA.

Epitope Identification. Two mice (No. 4 & 5) of each strain were selected (Before immunization, 2nd immunization, 4th immunization) for a PEPperPRINT® Chip assay that enabled analysis of reactivity to overlapping 13 aa linear peptides epitopes from HIV-1 JR-FL gp160.

Results

1. The heterogeneity of the anti-JR-FL gp140 response varied by D_H genotype with ΔD-DFL>Wild Type (WT)>>ΔD-DμFS, ΔD-iD.
2. Serum Ab using WT D_H bound best to the immunodominant epitope. Serum from ΔD-iD bound most strongly to selected epitopes associated with viral neutralization (Figure 3).
3. D_H-altered mice displayed altered recognition of gp140 linear peptides (Figure 4).
4. Serum Ab from ΔD-iD bound to peptides with a greater range of hydrophobicity, but the intensity of binding was lower than in the other mouse strains (Figure 5).

Conclusion

The pattern of epitope recognition and antigen binding in this T dependent response to a viral antigen depends on D_H gene segment sequence. Restrictions imposed by evolution on D_H sequence may contribute to the delay in producing neutralizing antibodies to HIV.

Acknowledgment

Thanks to 2015 CROI **Young Investigator Scholarship**. This project is supported by NIAID ***5R01AI090742***.

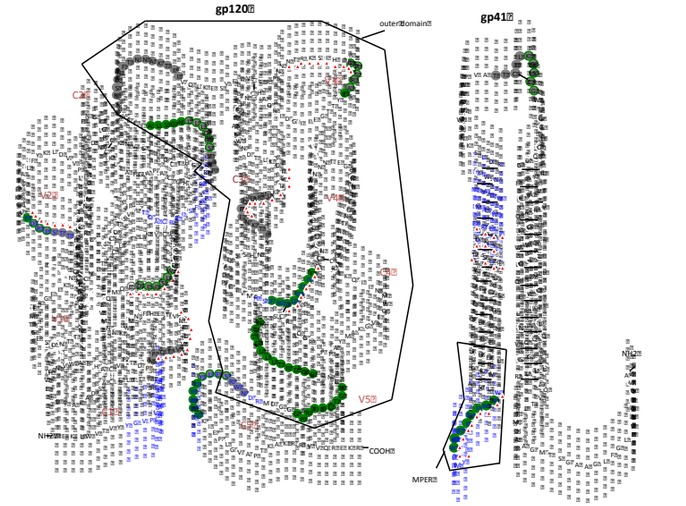


Figure 4. Illustration of the reactive peptides onside HIV-1 JR-FL 140. The reactive peptides were calculated by overlapping reactive peptide both in secondary and 4th immunization. The responsive residues on HIV-1 JR-FL envelope protein were highlighted by WT (Grey), DFL16.1 (Green), ΔD-DμFS (Blue), ΔD-iD (Red triangle), respectively. Depiction of JR-FL gp120 and gp41 structure were based on the results by Binley, et al (JM Binley, et al. *JVI* 2000 *Fig.3*).

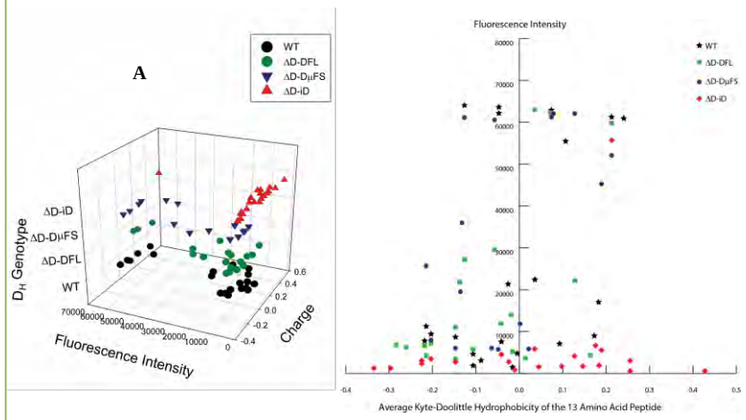


Figure 5. Responses of mice genotype versus intensity and charge of the reactive peptides. (A) 3-D visualization of the reactive HIV envelopes peptides responsive intensity with their charge and corresponding mice DH genotype. The plotting was done by SigmaPlot®. (B) Visualization of the mice strain preference, charge and reaction intensity in 2-D way. This plotting were completed by Adobe Illustrator.