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Poster presentation

P12-12. Analysis of antibody and B cell responses following inoculation with computationally designed HIV-1 2F5 epitope scaffold proteins

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Background

Two of the existing broadly neutralizing antibodies (NAbs) to HIV-1 Env, 2F5 and 4E10, recognize conserved continuous epitopes in the gp41 membrane-proximal external region. Re-elicitation of NAbs to the 2F5 epitope by immunization with peptides or chimeric proteins has so far proven difficult. To improve the efficiency of eliciting 2F5 epitope-directed NAbs, computationally designed scaffold proteins were used for more optimal presentation of the crystallographically defined 2F5 epitope (2F5E) to B cells. Here, we characterize 2F5E-specific B cell subsets and serum responses elicited by homologous and heterologous scaffold immunizations, with the overall goal to understand the rules of immuno-focusing in this novel system.

Methods

Computationally designed proteins, consisting of non-HIV proteins engrafted with the structurally defined 2F5E, were used in sequential immunizations to immuno-focus the humoral response on the 2F5E graft. A 2F5E-specific B cell ELISpot assay was developed and used for quantification of 2F5E-specific B cells in immunized mice. 2F5E-specific Ab levels in sera were analyzed by selected ELISA formats.

Results

A 2F5E-specific B cell ELISpot assay was optimized using a mouse hybridoma cell line specific for this epitope. Using this assay, we enumerated the number of 2F5E-specific antibody-secreting cells and memory B cells induced by homologous and heterologous scaffold immunization. Increases in the number of B cells specific for the 2F5 epitope were observed in groups of mice inoculated with heterologous scaffolds suggesting that the immuno-focusing strategy was successful. Increased Ab responses against the 2F5E were confirmed by ELISA.

Conclusion

By establishing a highly sensitive 2F5E-specific B cell ELISpot assay, we could quantify 2F5E specific B cell responses in mice immunized with different scaffold proteins presenting the 2F5E. Further analysis of the quality of the antibodies induced by homologous and heterologous scaffold immunizations is ongoing.