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Identification of an anti-gonococcal nanobody against the conserved 2C7 epitope

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Background

Treatment of gonorrhoea is difficult given the high degree of gonococcal surface variation. The identification of a conserved, phase invariant lipooligosaccharide epitope (termed 2C7) has therefore been of considerable interest as a target for therapeutic development. Not only has vaccination with a peptide mimic of this epitope enabled protective responses in mice, but a high affinity monoclonal antibody has also been raised. While the 2C7 monoclonal possesses an array of desirable traits for therapeutic use, stability at mucosal surfaces and intracellular penetration remain outstanding concerns. Here, we explore the generation of antibody fragments from camelid species, termed nanobodies, against the conserved 2C7 epitope. Nanobodies are significantly smaller than monoclonal antibodies and are much more soluble and stable – increasing their in vivo half life and ability to both enter and act intracellularly.

Aim/Methods

The aim of this project is to identify a high affinity anti-gonococcal nanobody against the conserved 2C7 epitope. Synthetic nanobody libraries generated by Zimmermann et al., (2020) were panned against the 2C7 mimotope (Octa-MAP1) identified by Ngampasutadol et al., (2006). Three libraries were screened, each with distinct interaction surfaces – concave, protruding, or a convex loop. Screening involved one round of ribosome-display, two rounds of phage-display, and single-clone expression ELISA. Throughout selection rounds, the surface chemistry for target immobilization was altered, minimizing non-specific binder enrichment.

Results

Out of the 382 clones assessed via crude ELISA from the convex and concave libraries, twenty-five demonstrated above background signals, with 76 % from the convex library. From these twenty-five hits, six candidates were selected based on signal extent and library distribution. All six hits displayed signal at least 85 % above background in their purified form, with one concave clone demonstrating a significant 388 % above-background average signal. Albeit with a reduced affinity compared to a 2C7 monoclonal antibody, this

top candidate demonstrates binding to both Octa-MAP1 and to gonococcal cell lysates.

Conclusions

To our knowledge, we have identified the first anti-gonococcal nanobody. Future work involves affinity maturation to maximize its use in novel therapeutics.