The use of human and mouse PBMCs to identifying monoclonal antibodies against virulence factors of streptococcus pyogenes

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INTRODUCTION

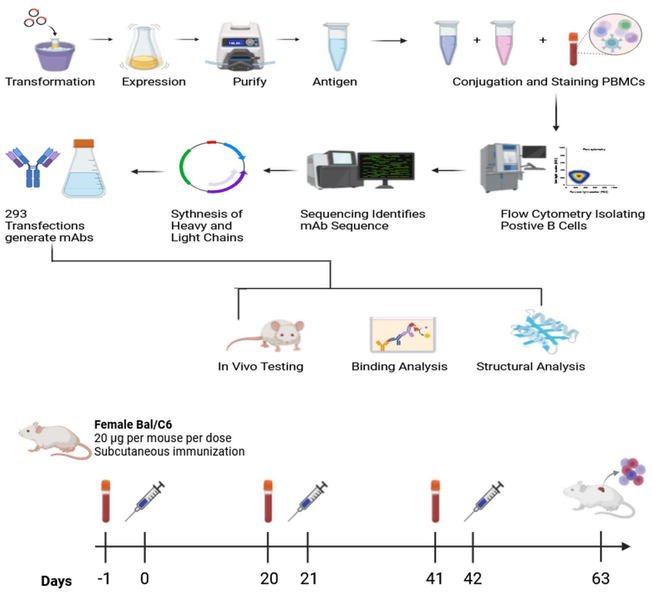
Streptococcus pyogenes, also known as Group A Strep, is a bacteria that causes many different human illnesses. While most people know it as the cause of "strep throat," it can also lead to deadly conditions like heart disease and toxic shock. Every year, this pathogen causes 40 million infections and 300,000 deaths worldwide.

Despite these high numbers, there is currently no vaccine available for Group A Strep. Current treatments rely on antibiotics, but these do not always prevent long-term damage. Our research focuses on creating monoclonal antibodies; specialized proteins that can target and stop the bacteria and to help scientists design a future vaccine.

METHODS

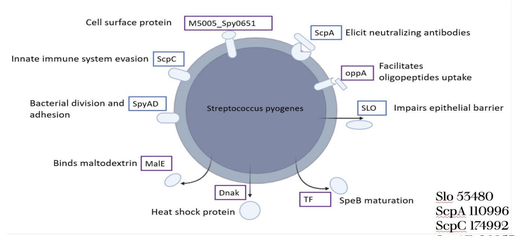
To find the right antibodies, we first had to "grow" specific parts of the bacteria, called antigens, in the lab. We chose nine different proteins, such as SLO and DnaK, because they are found on the surface of the bacteria. These proteins were purified to ensure they were clean and safe for testing.

We then used a process called "immunization" where mice were given small, safe amounts of these proteins. After 63 days, we collected their immune cells and used a machine called a Flow Cytometer to sort through millions of cells. This helped us find the specific "B-cells" that successfully created antibodies against our Strep A targets.

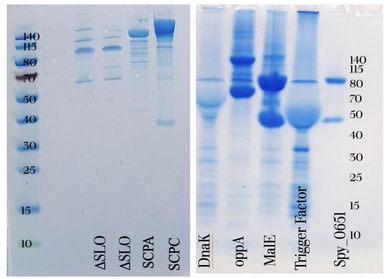


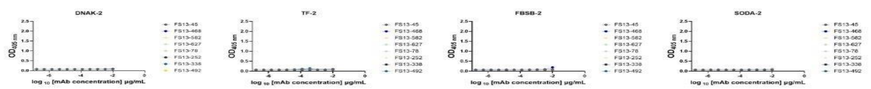
RESULTS

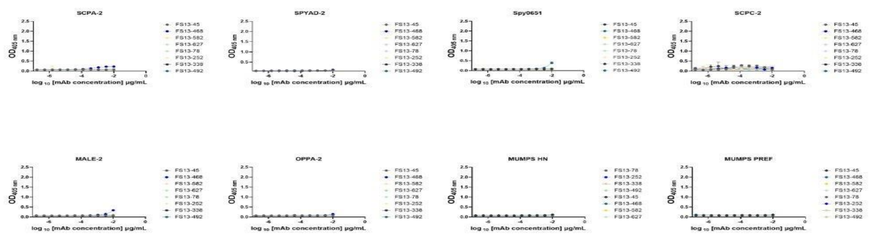
Our laboratory work successfully produced several key bacterial proteins, which we confirmed using a gel test that shows protein size. Specifically, we identified that one antibody, named FS13-492, showed an extremely high ability to bind to the SLO toxin.

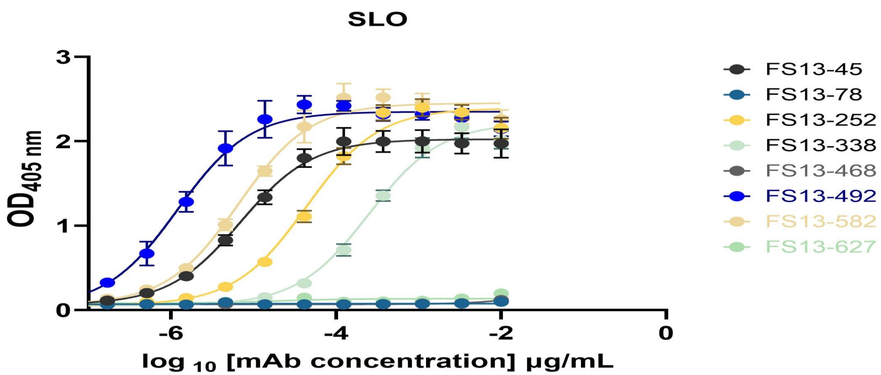


Slo 53480
ScpA 110996
ScpC 174992
SpyAD 26853
Dnak 64,788
MalE 44,455
OppA 71,902
TF 46,950
Spy_0651 417,298









CONCLUSION

This research successfully answered the question of whether we could create tools to target the proteins of *Streptococcus pyogenes*. By identifying antibodies like FS13-492 that bind strongly to the SLO toxin, we have found a way to potentially stop the bacteria from damaging human cells. This is highly significant because Strep A currently causes 300,000 deaths every year, and there is no vaccine to prevent these losses. Our work provides a specific blueprint for a vaccine that could finally offer protection against this global health threat.

While we have made great progress, this research is still ongoing. A major strength of our study was our ability to successfully purify many different bacterial proteins. However, a limitation we faced was that early cell sorts did not always yield results, and some proteins were very difficult to produce in large enough amounts for testing. These challenges show that finding the perfect antibody is a long and difficult process. We are working on testing the mAbs ablity to neutralize the toxic SLO protein.

	0.1	0.05	0.025	0.0125	0.00625	0.00315	0.00163	0.00081	0.00039	0.00019	9.77E-05	4.88E-05	Wavelength
SLO + FS13-338	0.108	0.128	0.158	0.114	0.116	0.11	0.1	0.1	0.114	0.084	0.174	0.084	410
SLO + FS13-252	0.067	0.072	0.068	0.071	0.075	0.074	0.073	0.079	0.074	0.075	0.077	0.072	410
SLO + FS13-45	0.06	0.061	0.062	0.061	0.064	0.064	0.064	0.064	0.064	0.061	0.161	0.054	410
SLO + FS13-582	0.061	0.061	0.059	0.059	0.062	0.062	0.067	0.066	0.061	0.066	0.066	0.072	410
SLO + FS13-492	0.061	0.063	0.06	0.058	0.06	0.066	0.075	0.059	0.146	0.065	0.063	0.063	410
.1% Triton X-100	1.863	1.833	1.817	1.836	1.924	1.873	1.887	1.885	1.779	1.807	1.811	1.809	410
SLO + FS13-78 (-)	0.058	0.059	0.06	0.07	0.064	0.059	0.064	0.061	0.068	0.066	0.092	0.074	410
BLANK	0.054	0.054	0.056	0.054	0.054	0.054	0.05	0.054	0.055	0.054	0.054	0.054	410

	1	2	3	4	5	6	7	8	9	10	11	12	Wavelength
ΔPLY	0.069	0.082	0.093	0.159	0.136	0.634	0.538	0.288	0.29	0.201	0.208	0.159	410
PLY	3.101	2.955	3.162	3.612	3.099	2.879	2.533	3.298	3.011	2.777	3.259	2.998	410
SLO	2.459	2.318	2.817	2.512	2.88	2.693	2.58	2.49	2.927	2.407	2.368	2.259	410
.1% Triton X-100	3.064	3.173	3.212	3.118	3.186	3.124	3.107	3.191	2.991	2.983	2.82	3.228	410
PBS	0.224	0.345	0.35	0.454	0.466	0.964	0.988	0.619	0.357	0.291	0.194	0.154	410
BLANK	0.054	0.055	0.054	0.055	0.055	0.054	0.054	0.054	0.055	0.055	0.055	0.055	410
BLANK	0.054	0.054	0.054	0.054	0.054	0.054	0.054	0.054	0.055	0.055	0.055	0.055	410
BLANK	0.054	0.054	0.054	0.054	0.055	0.055	0.054	0.055	0.055	0.054	0.055	0.055	410

We confirmed that SLO was able to lysis sheep red blood cells.

FUTURE DIRECTIONS

In the future, we will move beyond lab dishes to start animal studies to see if these antibodies can protect a living body from infection. We also plan to test a new group of antibodies from our most recent cell sorts to see if we can find even stronger options. We are currently producing more mAb identified in the human PBMC and mice PBMC flow sorts.

ACKNOWLEDGEMENTS

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