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Antigenic variation in Mycoplasma genitalium reduces antibody-antigen association kinetics

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ANTIGENIC VARIATION IN *MYCOPLASMA GENITALIUM* REDUCES ANTIBODY-ANTIGEN ASSOCIATION KINETICS

by

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HONORS ABSTRACT

The question of what makes humans *human* is one of philosophical discussion, but it runs the risk of bypassing the knowledge that humans are animals existing in a world filled with other organisms. These organisms interact with humans constantly. Considering that humans do not exist independently of other organisms, we are impacted by the evolutionary direction that they take. Therefore, if we have a desire to live alongside other creatures, humans have the responsibility to understand how other organisms intermingle with us. *Mycoplasma genitalium* is a sexually transmitted bacterium that can cause persistent infections lasting from months to years by using adhesin proteins to bind to the epithelial cells of the host's genital tract. Previous research on antigenic variation in the adhesin proteins MgpB and MgpC suggests that *M. genitalium* may use this variation to evade the immune system. Antibodies depend on specific binding to ensure that they connect to a particular pathogen with high binding affinity. It is suggested that the variation in the adhesin proteins creates a difference in the protein tertiary structure to the point the extent that antibodies no longer bind as well, allowing for the perseverance of the bacteria and persistence of the infection. We used surface plasmon resonance (SPR) to study the binding kinetics of antibodies from immunized rabbits and experimentally infected primates to recombinant MgpB protein fragments. We find that association kinetics of the MgpB:B variants were reduced when compared to the MgpB:B wild type.

ABSTRACT

Infections caused by *Mycoplasma genitalium*, a human genital tract pathogen, often persist for months to years. Antigenic variation of the immunodominant adhesin proteins, MgpB and MgpC, is thought to enhance persistence by avoiding specific antibody-dependent immune clearance. We used surface plasmon resonance (SPR) to study the binding of antibodies from immunized rabbits and experimentally infected primates to recombinant MgpB protein fragments. Primate antibodies collected from 2 weeks before to 2, 4, and 8 weeks after infection associated specifically with two different domains of the MgpB adherence protein (variable region B and a conserved C-terminal region) bound via amine coupling to the surfaces of CM5 sensor chips. Association and dissociation kinetics were measured and used to calculate dissociation constants (KD values). Association kinetics varied by 80-fold over the 14 antibody-antigen combinations tested, while dissociation kinetics varied by only 4-fold, suggesting that association kinetics describe the interactions better than dissociation.

In general, antibodies from later time points in the immune response bound antigens more tightly and more quickly, as expected from the process of affinity maturation. Antibodies bound best to the conserved C-terminal region, increasing in affinity from 300 nM to 5 nM for antibodies 4 or more weeks after infection. Binding to variable region B also increased from 300 nM to 5 nM, but highest affinity was measured 2 weeks after infection, after which affinity decreased. Binding was lowest to a variant B region that predominated 8 weeks after infection. Antibodies before infection bound with weak (1 μM) affinity, increasing to around 300 nM after infection. None of the antibody samples bound the variant B region with better than high-nanomolar affinity. Overall, we measured that antibody affinity increased by 7- to 20-fold after infection, but that this could be counteracted by sequence variation that reduced peak affinity by 2- to 3-fold.

INTRODUCTION

Mycoplasma genitalium, sporting the smallest genome of any known free-living organism (Glass, et al., 2005) can cause acute and chronic urethritis in men and likely cervicitis and pelvic inflammatory disease in women (Gnanadurai & Fifer, 2020). Along with urethritis, *M. genitalium* has been associated with more severe HIV and cancer development (Zarei, Rezania, & Mousavi,

2013). By adhering to the epithelial cells within the urinary tract, the bacterium enters the host and causes an inflammatory response. Despite the immune response, it has been shown that untreated infections can last for months to years. The chronic nature and incessant symptom presentation suggest that *M. genitalium* evades immune recognition.

The tip organelle of *M. genitalium* contains the two adhesin proteins, MgpB and MgpC (McGowin & Totten, 2017). The bacterium's genome contains regions homologous to the genes that code for adhesin proteins. Previous studies have shown that *M. genitalium* utilizes these homologous regions to generate recombinant proteins in within the tip organelle. With the many variable regions present within the adhesin proteins, crossed with the many homologous copies contained in the genome, there are many variants possible. The way in which the organism utilizes this recombination to evade the immune system is of great interest and is the focus of our research. Most antibiotics currently available work by targeting bacterial cell walls; without the cell wall, many bacteria lack the integrity to survive in the host. *M. genitalium* does not have a cell wall, so the antibiotics that are available to target it are minimal (Bradshaw, Jensen, & Waites, 2017). The available antibiotics that do work against *M. genitalium* are becoming futile as the percentage of antibiotic-resistant bacteria continues to rise. Successful treatment options depend on the understanding of how *M. genitalium* is able to avoid the host's immune response.

The recombinant regions of MgpB are B, EF, and G (Figure 1). We evaluated the kinetic signatures of antibodies from rabbits and primates with recombinant MgpB:B protein fragments. We used surface plasmon resonance to evaluate the association and dissociation kinetics of antibody-antigen binding at the intervals of 2 weeks prior to inoculation, 2 weeks after infection, 4 weeks after infection, and 8 weeks after infection. This was compared to the association and dissociation kinetics of antibody-antigen binding using the same intervals for a week 8 variant.

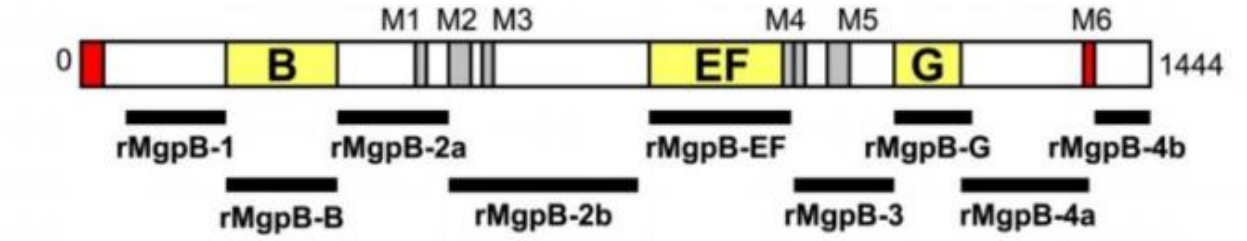
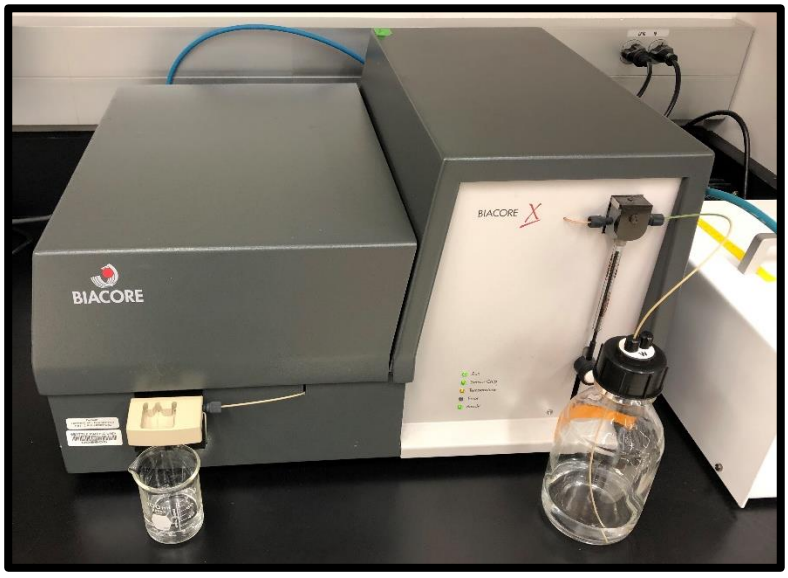


Figure 1: A diagram representing the variants present in the MgpB adhesin protein. Variable regions are in yellow and labeled B, EF, and G. rMgpB-B is the recombinant protein fragment we used (Iverson-Cabral, Wood, & Totten, 2015).

MATERIALS AND METHODS

We used a BIAcore X Surface Plasmon Resonance (SPR) instrument (Figure 2) to detect protein binding events. Antibodies were purified from sera of rabbits and primates infected with recombinant protein and *M. genitalium*, respectively. Three BIAcore chips were made by either amine-coupling MgpB:4a (a conserved region) protein fragment to the chip surface, amine-coupling MgpB:B:G37 (wild-type) protein fragment to the chip surface or amine-coupling MgpB:B Wk8 (variant) protein fragment to the chip surface. Varying concentrations of primate antibody from Wk-2, Wk2, Wk4, and Wk8 were run over the chips.



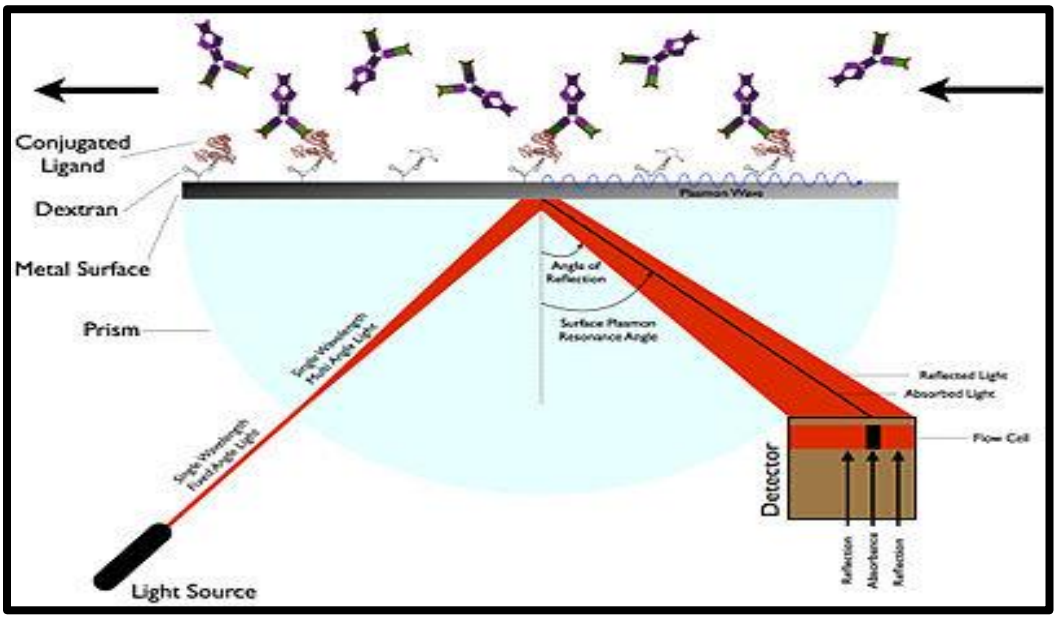


***Figure 2**: (Top) The Seattle Pacific University BIAcore X machine allows for collecting binding occurrences from SPR. (Bottom) SPR is able to express protein binding by measuring the change in the angle of reflection in a beam of light. As binding occurs, the angle of reflection is slightly altered. SPR allows for the change to be measured in real time. Image is taken from Sabban, Sari (2011 PhD thesis), The University of Sheffield.*

Surface Plasmon Resonance (SPR) Data Collection

General Information

All data collection was performed at 25°C using the same BIAcore X machine and BIAcore X control software. The machine was blanked using HBSEP buffer solution before samples were run and frequently during collection. Flow channel 1 was set as the blank flow channel, and all results are based on flow cell 2 – flow cell 1 values. Flow cell 1 did not have any antigen bound whereas flow cell 2 was set up to have antigen bound via amine-coupling. The machine was desorbed every Tuesday and sanitized the first Tuesday of the month during data collection. Desorption used BIAdesorb solution 1 (0.5% (w/v) sodium dodecyl sulphate) and BIAdesorb solution 2 (50 mM glycine at pH 9.5). Sanitization was performed using 7% of a 10-15% bleach solution.

Determining pH for Amine-Coupling

In order to combine the protein to the chip, the antigen needed to be charged for optimal binding. We used various pH solutions to test which pH would be best for the coupling reaction. The settings for the BIAcore machine were set at multichannel, rate of 10µL/min, and flow cell 1 used as the reference cell. We inserted a blank CM5 chip into the docking mechanism and allowed HBSEP to run flush through the inlet tube and onto the chip for a few minutes. We used sodium acetate at pH of 4.05, 4.51, 5.08, and 5.49 to dilute protein to 1:5. These samples were injected, and the 1:5 solution at pH 4.51 showed the best results for coupling by showing the highest response difference present in flow cell 2.

Amine-Coupling

Amine-coupling is necessary to bind the protein of interest to the surface of the chip. The first step is to activate and block the blank surface (flow cell 1). The settings for this were single-channel, flow cell1 as reference, and the rate was 10 µL/min. The flow cell surface was cleaned

with a 10 µL (35 µL) solution of 50 mM NaOH. We then injected 25 µL (60 µL) of a 1:1 NHS:EDC mixture, and we finished the cleaning with an injection of 35 µL (60 µL) ethanolamine solution. We then needed to activate, couple, and block the flow cell 2 protein surface. This was done by setting the machine to single-channel, reference cell 2, and a baseline flow to 10µL/min; we started a continuous flow of HBSEP. The flow cell was set to 2 so that our antigen only bound in that channel. We injected 25 µL (60 µL) of a 1:1 solution of NHS:EDC (N-hydroxysuccinimide at a concentration of 0.1 M in water and 0.4 M 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride in water). We then injected 80 µL (110 µL) of the protein:acetate solution (a 1:5 solution of 15 µL antigen (MgpB:C4a-1, MgpB:B G37 or MgpB:B Wk8 variant) and 85 µL pH 4.51 acetate). A 25 µL (50 µL) ethanolamine solution was injected to block any remaining areas to bind to reduce false binding responses.

Regeneration of Surface

Previous research on this project has utilized NaOH as a method of regenerating the chip surface. We found that the binding events of the primate antibodies were weaker than the previous year's rabbit antibodies, and the wash procedure that occurred after the sample had been run through was sufficient in removing any extra analyte.

Antibody Concentrations

Primate A01220 Antibody Concentrations	
Wk-2	0.913 mg/mL
Wk2	0.939 mg/mL
Wk4	0.961 mg/mL
Wk8	0.746 mg/mL

Table 1: *Original concentrations of primate A01220 antibodies from various weeks.*

To ensure that the concentrations, once diluted, were comparable between the G37 chip and the Wk8 variant chip, dilutions were made to get as close to a similar concentration as possible. The concentrations used on the Wk8 variant chip were doubled due to reduced binding activity. Antibodies were mixed with HBSEP buffer to achieve desired concentration.

Primate A01220 Antibody used on MgpB:B G37 Chip	Concentration Range	Primate A01220 Antibody used on MgpB:B Wk8 Variant Chip	Concentration Range
Wk-2	1:608, 1:304, 1:152, 1:76, 1:38, 1:19	Wk-2	1:304, 1:152, 1:76, 1:38, 1:19, 1:9.5
Wk2	1:640, 1:320, 1:160, 1:80, 1:40, 1:20	Wk2	1:320, 1:160, 1:80, 1:40, 1:20, 1:10
Wk4	1:640, 1:320, 1:160, 1:80, 1:40, 1:20	Wk4	1:320, 1:160, 1:80, 1:40, 1:20, 1:10
Wk8	1:496.64, 1:248.32, 1:124.16, 1:62.08, 1:31.04, 1:15.52	Wk8	1:248.32, 1:124.16, 1:62.08, 1:31.04, 1:15.52, 1:7.75

Table 2: *Concentration gradients used during data collection for both the G37 and Wk8 variant chips.*

Ligand Placed on Chip Surface	Initial Concentration Received
MgpB:C4a-1	1.00 mg/mL
MgpB:B G37	0.32 mg/mL
MgpB:B Wk8	0.59 mg/mL

Table3: *Antigen initial concentrations that were used to make the chips.*

Data Collection

All trials followed the same procedure for collecting data. BIAcore was set to multi-channel, FC2-1 run with flow cell 1 used as reference, and a flow rate of 40 µL/min. The wash was set for a 180 second delay. The first injection was a 60 µL (90+5+5+5 µL) HBSEP solution. Once the baseline had steadied, concentrations (shown in Table 2) were made and injected using the volume 60 µL (90+5+5+5 µL). After each cycle, an additional 250 seconds were left between the samples to allow the baseline to return to the initial position. Four trials were taken for each sample.

Data Processing

BIAevaluation was used to process the data of the binding curves. Each trial was analyzed individually for the kinetic data. We used a 1:1 Langmuir binding model to fit both the association and dissociation kinetics. From this, we were able to determine the k_{on} and k_{off} values. With these values, the overall KD value was determined (Figure 3).

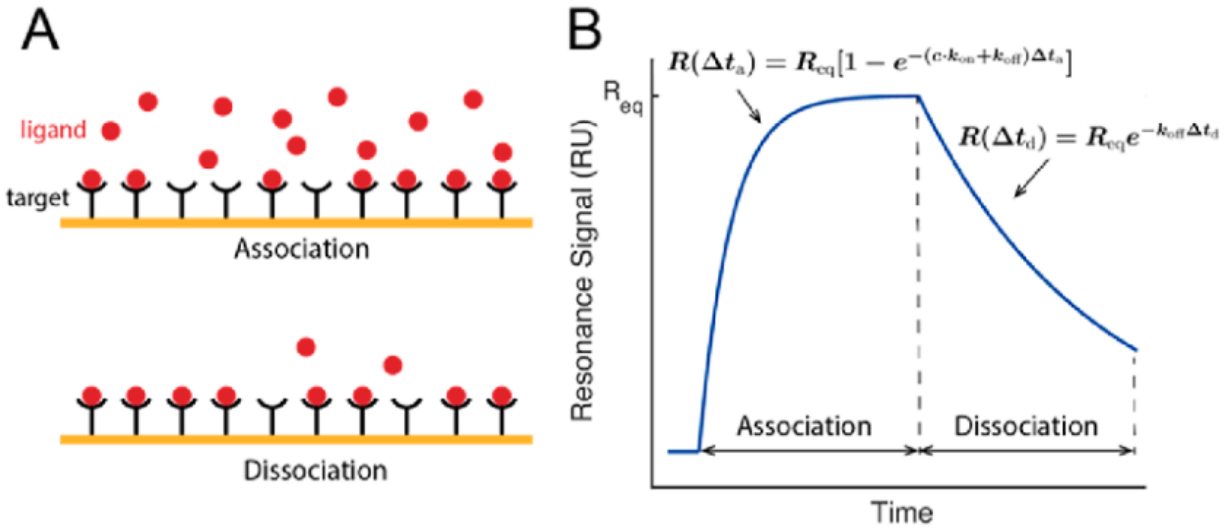


Figure 3: *Figure taken from Wang, Yan, and Goult's paper on force-dependent binding constants* (Wang, Yan, & Goult, 2019). *A shows the act of ligands binding and then dissociating. B shows the graphical representation of the association and dissociation occurring on the chip. The equations given can be used to calculate kinetic constants using BIAevaluation software.*

RESULTS AND DISCUSSION

The two regions studied during this project were MgpB:B and MgpB:C4a-1 with the former tested with the G37 wild type and a Wk8 variant. Figure 4 demonstrates the regions in which these fragments make up the MgpB adhesin protein.

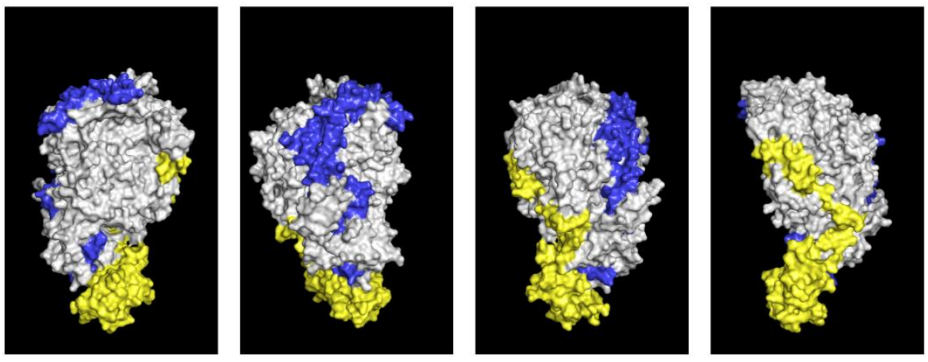


Figure 4: *Using PYMOL and the PDB ID 6RUT, the 3D structure of MgpB can be visualized with regions B (blue) and C4a (yellow) contributing to much of the surface.*

Our findings show that dissociation rates do not change much between the G37 wild-type and the Wk8 variant as the dissociation kinetics only varied about 4-fold (Table4).

ka	SE(ka)	kd	SE(kd)	Chip (Antibody)	KD
16043.67	5659.333	0.00395	0.000462	4a(wk-2)	2.46E-07
22280	8891.708	0.008226	0.001093	4a(wk2)	3.69E-07
122339.8	21855.45	0.004822	0.000725	4a(wk4)	3.94E-08
64566.67	13231	0.003532	0.000449	4a(wk8)	5.47E-08
11410	5232.278	0.003823	0.000677	B(Pwk-2)	3.35E-07
90993.89	35357.22	0.004794	0.001069	B(Pwk2)	5.27E-08
32868.33	17344.44	0.007477	0.001361	B(Pwk4)	2.76E-07
12444.17	5535.333	0.004191	0.000973	B(Pwk8)	3.37E-07
1492.783	996.4833	0.002618	0.000244	Bwk8(Pwk-2)	1.75E-06
18423.61	6254.167	0.003044	0.000579	Bwk8(Pwk2)	1.86E-07
30191.46	12774.17	0.011513	0.002859	Bwk8(Pwk4)	3.81E-07
12227.08	3847.917	0.004565	0.000513	Bwk8(Pwk8)	3.73E-07
72850	1511.25	0.002678	0.000156	B(G37)(rab)	3.68E-08
44350	2643.5	0.003063	0.000344	Bwk8(rab)	6.91E-08

Table 4: *Kinetic binding for k_a and k_d with total K_D. Binding affinity ranges from tight (green) to weak (red). Rab stands for rabbit primates that were studied prior to the primate antibodies.*

The dissociation kinetics vary by 4-fold whereas the association kinetics 80-fold; the association kinetics describe the interaction between bacterium and antibody better than the dissociation. The primate antibody flown over the MgpB:C4a-1 chip demonstrated increased binding from Wk-2 to Wk4; association binding began to decrease by Wk8 (Figure 5). Primate antibody interacted weakly with the MgpB:B chip during Wk-2 and increased to its tightest binding during Wk2. It steadily decreased through weeks 4 and 8 (Figure 6). The lowest binding was seen between the primate antibody and the MgpB:B Wk8 variant chip with extremely low binding during Wk-2 and steadier binding until Wk4, when binding became weaker for Wk8 (Figure 7).

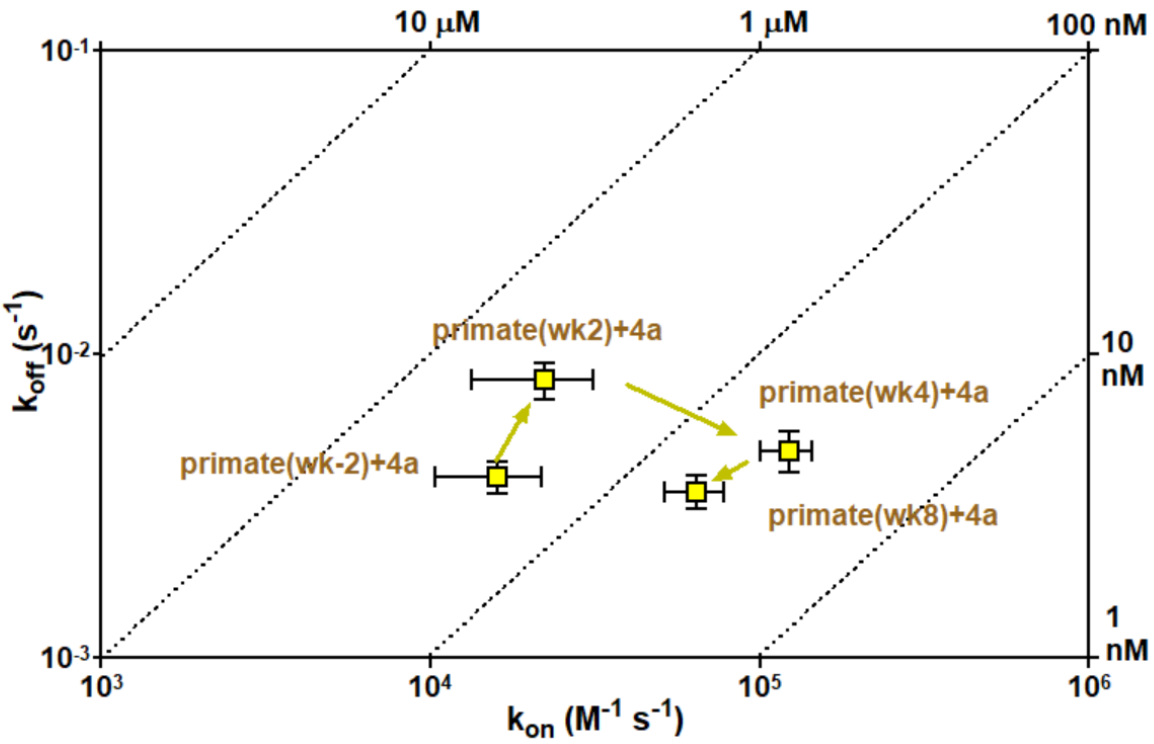


Figure 5: *k_{on}-k_{off} plot for the group of tests done between primate antibodies (from 2 weeks before inoculation to 8 weeks post inoculation) run over an MgpB:C4a-1 chip. Between weeks -2 and 4, association binding gets stronger. Between weeks 4 and 8, the association between antibody and antigen weakens.*

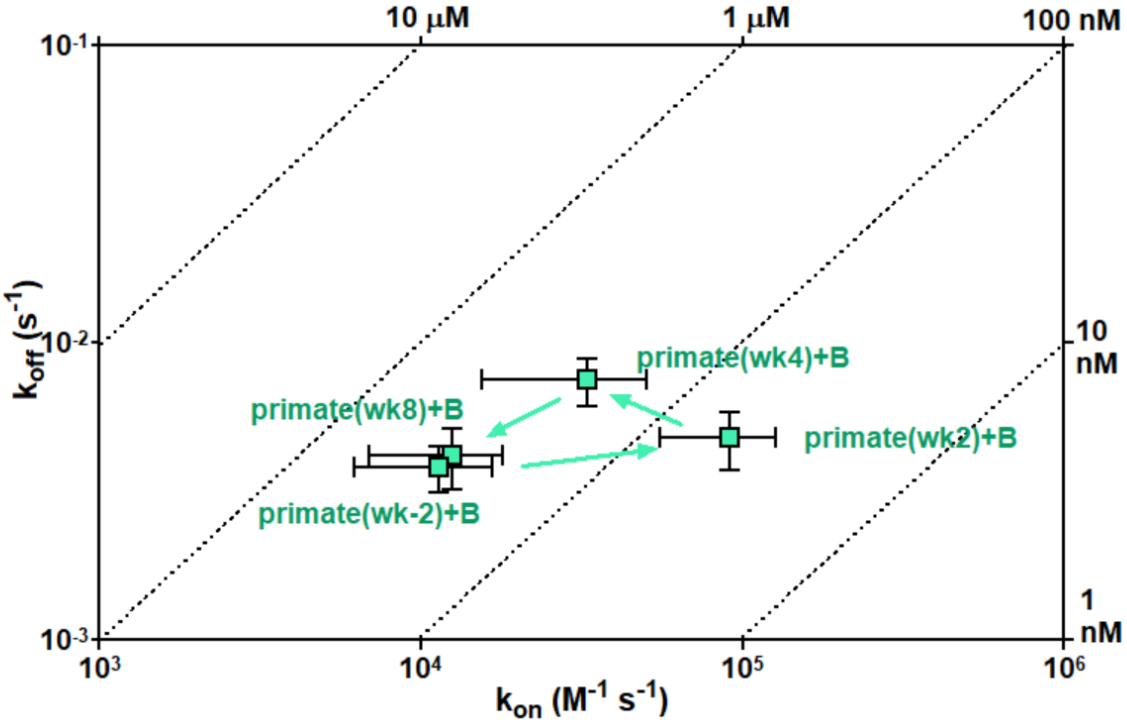


Figure 6: *k_{on}-k_{off} plot for the group of tests done between primate antibodies (from 2 weeks before inoculation to 8 weeks post inoculation) run over an MgpB:B. Between weeks -2 and 2, association binding gets stronger. Wk4 antibodies bind less strongly than Wk2, and Wk8 antibodies show a similar affinity for the antigen as antibodies that have never been exposed to MgpB:B.*

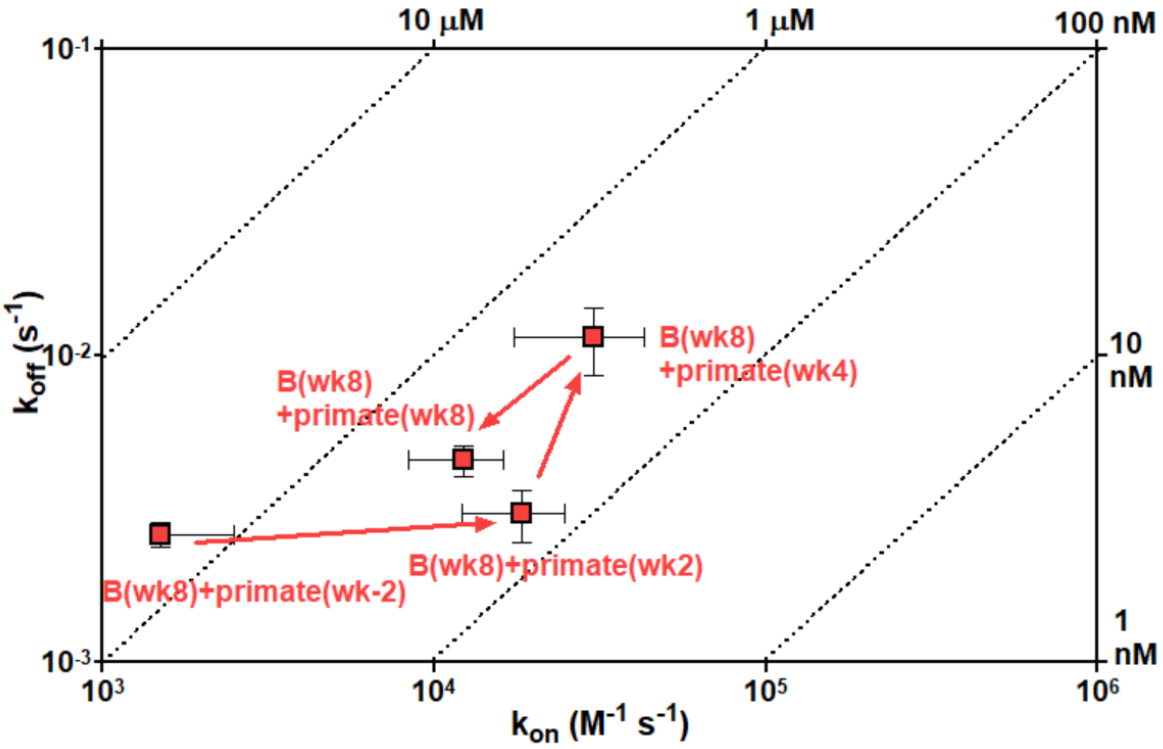


Figure 7: *k_{on}-k_{off} plot for the group of tests done between primate antibodies (from 2 weeks before inoculation to 8 weeks post inoculation) run over an MgpB:B Wk8 variant chip. Wk-2 showed incredibly low affinity for the antigen, even lower primate(wk-2)+B in the previous figure. The highest association occurred with the Wk4 antibodies.*

Our findings also show that the variation leads to reduced antibody-antigen association kinetics. The association kinetics of the 14 combinations tested varies by 80-fold. The general finding is that antibodies bound better later during the infection. Studies on region B show that the highest affinity, however, was during 2 weeks after infection. After those two weeks (as shown by Wk4 and Wk8), binding affinity decreased. The results of antibody being bound to Wk8 variant antigen showed much lower binding affinities and a peak affinity during Wk4 (which was similar to the binding of Wk4 for the G37 antigen). It is important to note that the binding between the BWk8 variants and the Wk-2 antibodies is almost not detected. When compared to

the better binding of BG37 to the Wk-2 antibodies (antibodies that have never seen this antigen), it suggests that the region is varied so as to not bind antibodies in general, not just specific antibodies.

CONCLUSION

Understanding how *Mycoplasma genitalium* evades the immune response of the host is imperative to generating successful treatment plans. Understanding how *M. genitalium* evades the immune response *in vitro* is a necessary step in that process. Recognizing how variation within the *M. genitalium* genome can affect association and dissociation kinetics can provide insight into how the surface of the protein (in this case the tip organelle) affects the ability of the immune response. Our data show that these variations are affecting the initial binding between antigen and antibody suggesting that an alteration in the surface of the protein is preventing antibodies from binding. Future work on other protein fragments in the MgpB genome as well as the protein fragments of the other adhesin protein, MgpC, will help in determining how variation allows this bacterium to continue to evade the immune system. We hope to continue work using human sera on the recombinant proteins that have studied here as well as the others in MgpB and MgpC.

REFERENCES

Bradshaw, C. S., Jensen, J. S., & Waites, K. B. (2017). New horizons in Mycoplasma genitalium Treatment. *The Journal of Infectious Diseases*, *216*(suppl_2), S412–S419. https://doi.org/10.1093/infdis/jix132

Glass, J. I., Assad-Garcia, N., Alperovich, N., Yooseph, S., Lewis, M. R., Maruf, M., … Venter, J. C. (2006). Essential genes of a minimal bacterium. *Proceedings of the National Academy of Sciences*, *103*(2), 425–430. https://doi.org/10.1073/pnas.0510013103

Gnanadurai, R., & Fifer, H. (2020). Mycoplasma genitalium: A Review. *Microbiology*, *166*(1), 21–29. https://doi.org/10.1099/mic.0.000830

Iverson-Cabral, S. L., Wood, G. E., & Totten, P. A. (2015). Analysis of the Mycoplasma genitalium MgpB Adhesin to Predict Membrane Topology, Investigate Antibody Accessibility, Characterize Amino Acid Diversity, and Identify Functional and Immunogenic Epitopes. *PLOS ONE*, *10*(9). https://doi.org/10.1371/journal.pone.0138244

Lunn, Meg et al. (2020). The Kinetic Signatures of Antibody Binding to M. Genitalium Adhesin Protein Fragments. *Student Honors Project for Seattle Pacific University Honors Program*, 1-15.

McFarland, B. J. (2018). Measuring Novel Protein-Protein Binding with Surface Plasmon Resonance in the Physical Chemistry Lab. *ACS Symposium Series*, 15–31. https://doi.org/10.1021/bk-2018-1279.ch002

McGowin, C. L., & Totten, P. A. (2017). The Unique Microbiology and Molecular Pathogenesis of Mycoplasma genitalium. *The Journal of Infectious Diseases*, *216*(suppl_2), S382–S388. https://doi.org/10.1093/infdis/jix172

Wang, Y., Yan, J., & Goult, B. T. (2019). Force-Dependent Binding Constants. *Biochemistry*, *58*(47), 4696–4709. https://doi.org/10.1021/acs.biochem.9b00453

Zarei, O., Rezania, S., & Mousavi, A. (2013). Mycoplasma genitalium and Cancer: A Brief Review. *Asian Pacific Journal of Cancer Prevention*, *14*(6), 3425–3428. https://doi.org/10.7314/apjcp.2013.14.6.3425

APPENDIX

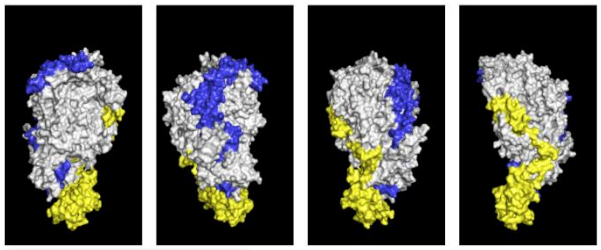
Our panel's discussion on the responsibilities of humans will work its way up from the micro to the macro duties that present themselves in our lives. The driving point of this discussion will be to recognize that we do not exist independently. We do not exist independently of other citizens. We do not exist independently of our peers. We do not exist independently of other organisms. Beginning with the micro responsibilities of humans, it is imperative to recognize that we do not exist in our bodies alone. The human body contains its own ecosystem of microbes. Some of these will be working symbiotically with us while others are with us purely for their own gain, using us as a mere means for their own benefit.

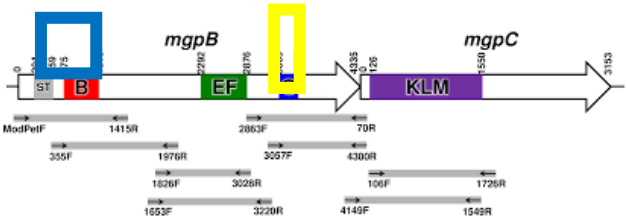
One such microorganism is *Mycoplasma genitalium*. This bacterium exists with a size of about 300nm and a genome consisting of 482 genes. For reference, this the smallest genome on record. *M. genitalium* is sexually transmitted, and I would be remiss if during a discussion on human responsibility and a cause of STDs that I didn't point out the duty to be responsible during physical relations. MG can affect both men and women. In men there will be cases of urethritis. In women, there will be cases of cervicitis, pelvic inflammatory disease, preterm birth, infertility, and an increased acquisition and transmission of HIV. Prior endeavors to combat bacterial infections gave rise to antibiotics like penicillin. Unfortunately, penicillin is only useful against bacteria that contain a cell wall, something that *M. genitalium* lacks. There are antibiotics that could work against MG, specifically azithromycin and moxifloxacin. Azithromycin works by inhibiting bacterial protein synthesis. Without proteins, the cell cannot function. Moxifloxacin is a fluoroquinolone that interferes with DNA gyrase during DNA synthesis within the bacterium so that it cannot replicate. However, MG infections are becoming increasingly resistant to the present options. Now we have the task of finding new treatment options for this bacterium or else be infected indefinitely. Some people who have the resistant strains have been infected for years. They depend on scientists to understand how this bacterium evades its attackers so that it can be targeted and the infection it causes can be stopped.

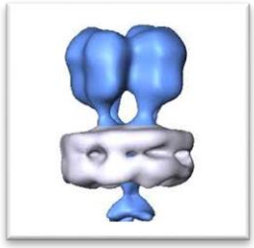
M. genitalium, though, does not want to be stopped. Along with the antibiotics that it has had to overcome, MG infections have to fight off the body's own defense mechanism: the immune system. After a pathogen has found a way past the first barrier, whether through the skin, eyes,

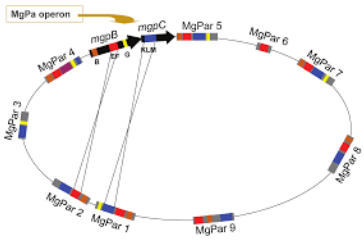
nose, or urogenital openings, macrophages work to consume the invading bacteria. Once they recognize and consume a threat, they can send out signals to neutrophils which will come to the location and secrete toxins to kill the bacteria. Dendritic cells will also get involved. Dendritic cells are known as APCs or antigen-presenting cells. They will take in the pathogen, break it apart, and present sections of it on its cell surface. Bacterial pathogens will be taken to the lymph nodes where there will be a T-cell that can recognize the antigen presented on the dendritic cell. While some of those T cells will stay in the lymph node for later immunity and some will go to the site of infection, others will continue on to the center of the lymph node and find a matching B cell. These B cells will make antibodies specific to the antigen. The antibodies will bind specifically to their targeted region either blocking the pathogen from entering the cell or recruiting cells and molecules to kill the bacterium.

Just as the human body is working to combat this infection, the bacterium is working to avoid









being killed. Within the entire genome of MG, there are two important proteins, the adhesin proteins. MgpB and MgpC make up the proteins found on the tip organelle of MG. This tip organelle is what allows MG to bind to epithelial cells found in the reproductive tract and get inside the body. The pictures above the adhesin proteins are the prote in crystal structure of MgpB taken from 4 different angles. I colored the structure based on the regions that my research focused on which I will get into a bit later. The yellow region is called 4a and the blue region is called B. The picture on the bottom right is the entire *M.* genitalium genome with the MgPa operon and MgPar regions labeled. The operon region is the region responsible for expressing the actual protein. These par regions contain the genetic information for the protein, but they are not being expressed. The picture in the top right is a closer look at the operon region. The genes of the unlabeled proteins are shown in white and are conserved regions. You'll also notice that there are certain regions

labeled as B, EF, G, and KLM. These are specially labeled because they are known as variable regions. As I said previously, around the chromosome will be Par regions that contain the DNA of these variable regions. What previous studies have shown is that homologous recombination occurs to cause a different protein sequence to be expressed. The full or partial recombination between the par regions and the expressed site leads to tremendously different possibilities every time the cell undergoes meiosis. What this means is that the operon is initially expressing the original B region. During replication, the organism may use homologous recombination to swap out that B region with a new B region found in one of the Par regions. There are 9 par regions containing different regions of the adhesin proteins. These can be recombined fully or partially which is why so much variation is possible within a population of MG.

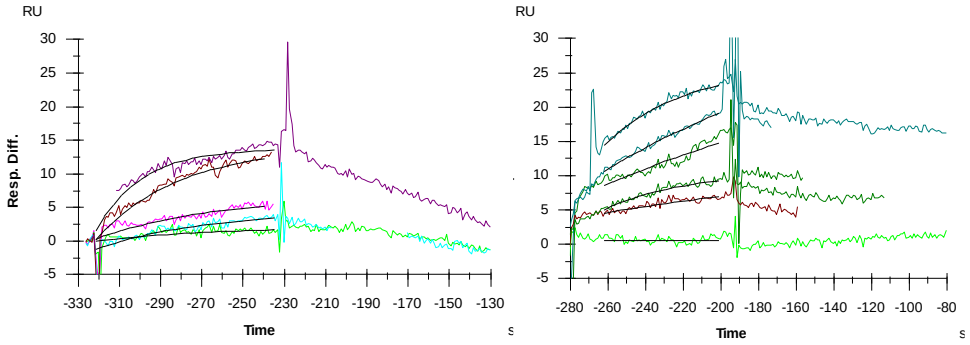
This is where my research on antibody binding came in. Knowing that *M. genitalium* can vary its expressed protein within the variable regions, our general question to answer was how those variants alter binding with the antibodies designed to bind to them. The antibodies that I used were taken from infected primates and given to me by Dr. Gwen Wood after purification by Laarni Aguila. Laarni also purified protein fragments of the adhesin protein MgpB. The regions that I tested were 4a, a conserved region, and region B, a variable region. I also received a variant of the B fragment. I will refer to this variant as the week 8 variant because it appeared in the bacterial population 8 weeks after the initial infection. This means that after 8 weeks of an infection, the bacteria that the immune system was fighting were slightly different than those that initially began the infection. The variation that occurred in the week 8 variant totaled only a few different bases, but this was enough to generate different amino acids. One you change the secondary structure of a protein, that is to say, once you change the building blocks that it's made of, you change its shape. Different shapes, even microscopic changes in the overall shape, lead to different molecular interactions between antibody and antigen and lead to different binding. Chemists will often say that when you change the shape you change the function. In this case, the evidence suggests that changing the shape changes the binding. To understand how these fragments influence antibody-antigen interactions, I used a method called SPR to analyze the binding events between the antibodies and their respective antigens. Once I understood how the

antibodies were binding, I was able to compare the different regions and understand what was happening to allow infections to persist.

Surface plasmon resonance is a method to collect real-time information on the interactions between biomolecules. It is an important technique to understand interactions between compounds because it gives insight into the kinetics of an interaction rather than just the affinity. The general idea is that the machine shines a polarized laser into a surface. We use a gold surface because it can conduct well. This will create plasmons which are electron charge density waves. The reflected light's intensity is reduced due to the plasmons, and it is reflected back at a specific angle called the resonance angle. This angle is proportional to the mass present on the sensor surface. This allows us to tell the difference between a chip's surface that only contains antigen and a chip's surface that is holding antigen bound to antibody because the angle of reflection will change. I used a method called amine-coupling that allows me to bind a protein to the surface and block the rest of the chip's surface from binding by flowing it over the chip's surface. The protein fragments that we used were MgpB:4a, a fragment of a conserved region in the MgpB adhesin protein, MgpB:B, a variable region in the MgpB adhesin protein, and MgpB:B Week8, which was the week eight variant of the MgpB:B fragment. Once I had confirmed that my ligand has bound to the chip, I was able to flow different concentrations of antibodies over the chip.

The antibodies that I received were taken two weeks prior to infection, two weeks post infection, four weeks post infection, and eight weeks post infection. I made serial dilutions of antibodies so that I could gauge how concentration affected binding and so that the antibodies from different weeks would have the same relative concentration when I tested them. I then ran four trials of each week's concentration gradient over all three chips. The chip is docked in the BIAcore machine with the protein fragment attached. I then pipette a small volume of dilute antibody into the machine where it will flow over the chip. The light that is being reflected off the chip will sense when antibodies from my sample initially bind as well as when they dissociate from the chip. The resulting curve allows me to calculate the k_{on} and k_{off} rates. The k_{on} rates describe how easy it is to form the initial interaction. The k_{off} rates describe how easy it is to break the interaction. Something with a high on and off rate wouldn't necessarily be great for an

important binding interaction because it would dissociate as soon as the two compounds came together. The first half or so of the graph is tracking the response difference as the antibodies are flowing over the chip. Response units increase as antibodies bind, causing the mass on the chip to increase and the angle of reflection to change. After the sample is done running over the chip, there is a flushing of HBSEP solution, which is a salt solution that we use to clean the machine. This is the same solution that I used to make dilute antibody samples and used to blank the instrument initially, so any binding between the HBSEP salts and the chip will not be reflected in the graph. The declining line seen in the graph is expressing the antibodies falling off the antigen. From this, I can calculate both the association and dissociation rates.



ka	SE(ka)	kd	SE(kd)	Chip (Antibody)	KD
16043.67	5659.333	0.00395	0.000462	4a(wk-2)	2.46E-07
22280	8891.708	0.008226	0.001093	4a(wk2)	3.69E-07
122339.8	21855.45	0.004822	0.000725	4a(wk4)	3.94E-08
64566.67	13231	0.003532	0.000449	4a(wk8)	5.47E-08
11410	5232.278	0.003823	0.000677	B(Pwk-2)	3.35E-07
90993.89	35357.22	0.004794	0.001069	B(Pwk2)	5.27E-08
32868.33	17344.44	0.007477	0.001361	B(Pwk4)	2.76E-07
12444.17	5535.333	0.004191	0.000973	B(Pwk8)	3.37E-07
1492.783	996.4833	0.002618	0.000244	Bwk8(Pwk-2)	1.75E-06
18423.61	6254.167	0.003044	0.000579	Bwk8(Pwk2)	1.86E-07
30191.46	12774.17	0.011513	0.002859	Bwk8(Pwk4)	3.81E-07
12227.08	3847.917	0.004565	0.000513	Bwk8(Pwk8)	3.73E-07
72850	1511.25	0.002678	0.000156	B(G37)(rab)	3.68E-08
44350	2643.5	0.003063	0.000344	Bwk8(rab)	6.91E-08

My overall data is described by this table. The colors demonstrate the binding affinities with green showing tight binding and red showing weak binding. Starting with the chip holding the 4a fragment, we see that binding is strongest with the week 4 antibody. This is also the strongest overall binding during an association event out of any antibody week over any protein fragment. The week 2 sample against the B fragment has the greatest association and affinity for either B fragment. This is expected due to affinity maturation which is the process by which B cells will create antibodies with increased affinity for their antigen over time. As the host is exposed to more pathogen, the host will make antibodies with better binding. However, we then see a decline in binding rather than a continual increase. With both B fragments, their association kinetics are remarkably lower than the 4a fragment. Considering that the 4a fragment is of a conserved region and the B fragment is of a variable region, it would make sense that the body would choose to make antibodies for a conserved region rather than a variable region. The B region changes over time based on the variants that it can use during homologous recombination. Therefore, the immune system is in a reactive stage with the B region rather than a proactive stage like with the 4a region. In this study, we are using chips bound with specific regions of *M. genitalium* for antibody targeting. The antibodies that we collected were from primates who were infected with the entire organism, not just a segment of the adhesin protein. This means that the purified antibodies we used to conduct our studies would have contained antibodies specific to region 4a, region B, region EF, region G, region KLM, and all of the regions not associated with the adhesin protein. Those antibodies would not necessarily be present in the same concentrations. In this case, the week 4 antibody sample that I ran over the region B chip may have contained more 4a antibody than B antibody. This could explain why we see lower levels of binding. Antibodies are made based on how well their B cell can be activated. So, all of this is to say that we suspect that our antibody samples contained more antibodies specific to region 4a than region B. Looking at our data in the broader sense, that means our body is not treating each protein fragment the same---it is learning which antibodies to make based on what is effective.

An interesting finding within the data for the B week 8 variant fragment is the extremely low association kinetics for the week -2 antibodies. Looking at the three graphs of the different chips

next to each other, we would expect that initial binding affinity and k_{on} rates to be similar over all of the chips for the week -2 antibodies. Afterall, the week-2 antibody samples were sera taken two weeks before the initial MG infection. Those primates had never been infected by MG, so there would be no reason for them to have any MG antibodies. Therefore, we would expect antibodies taken from this time-period to bind to the 4a, B, and B week 8 variants with about the same rate. However, instead we see much lower binding in the variant. This suggests that the variant that the bacterium uses affects general antibody binding, not just specific antibody binding. Our antibodies look generally about the same. They are a bendy Y shape that contain variable and constant regions. The variable regions are what allow such specific binding to a specific pathogen when presented with a plethora of things to attack. However, it does not seem as though the MG variant is affecting just the specific binding. Rather, it seems that the variant causes antibodies in general to not bind as well. Later on, we still see weak binding with antibodies from weeks 2, 4, and 8 within the B week 8 variant. This shows that after affinity maturation, there is still lower binding. Despite having had enough time for the antibodies to show stronger binding, they are still unable to bind as well as either the normal B fragment or the 4a fragment. So, while the week 8 variant lessens general antibody binding, it is also lessening specific antibody binding.

Something to note is that once we had collected all of our data with 4 trials of each antibody over the three chips, the association kinetics varied 80-fold. The dissociation kinetics varied 4-fold. So, while the table that I showed contained results of the dissociation kinetics, because they varied inconsequentially compared to the association kinetics, we felt that the association kinetics were a better representation of the antibody-antigen interactions occurring due to variance. We also calculated the total KD. KD is the equilibrium dissociation constant, so a ratio between k_{off} and k_{on}. It is inversely related to the affinity, and it relates to the concentration of antibody necessary for a certain level of binding. When describing this, we can use the terms micromolar, high-nanomolar, and mid-nanomolar. Since KD is inversely related to affinity, the higher the KD, the lower the affinity between the antibody and antigen. Most of the 4a fragment results were in the region of mid-nanomolar, meaning on the higher end of affinity. The B fragment interactions were typically in the high-nanomolar range. Only the B variant for the

week-2 antibody presented in the micromolar region, showing just how low the affinity was for that interaction. We can take these results and boil it down to strongest binding between antibody and antigen in region 4a, the conserved region. Second strongest binding in region B, a variable region. Weakest binding in the week 8 variant of the B region.

The historical timeline of this project began with similar studies on rabbits. The rabbits were infected with, rather than the entire organism, just the protein fragments. This allowed for the understanding of the kinetic binding of protein fragments to only their specific antibody as well as which protein fragments resulted in the highest and lowest affinity binding. From that knowledge, my project on primate antibodies came to be. The importance of my research in terms of an intermediate project was to show that binding could be detected at levels well enough to show existing differences. The original antibody concentrations that I received were already quite low, and I would then dilute the antibodies to around 1:1000th. Showing that data could be collected with such low concentrations was imperative before moving on to humans. Continuing with human trials would not yield desirable results if we were unable to visualize binding with the primate antibodies. Likely, this research will continue on with human antibodies as well as different protein fragments, both of the conserved nature and different variable regions. This knowledge will aid in designing more specific therapeutics in the future that can target the regions most important for bacterial survival. The antibiotic resistance of this bacterium continues to raise concern, and knowing that the conservative regions are the highest targets for antibodies gives new information on what therapeutics should target next.

Later on, in this panel we will hear about interactions between teachers and students and political leaders with their constituents. In each case we are dealing with humans interacting with one another, either in a small setting or on a large scale. While these interactions are at the forefront of our mind when we think about all of our daily interactions, there are a million cells within our body, both foreign and domestic, that are demanding our attention as well. *M. genitalium* is just one example of an organism requiring our consideration. As soon as we disregard its abilities or potential impact, it can change its protein expression causing a raging infection that cannot be ignored. There is a necessity of awareness to the world around us. In Christian theology, humans are called upon to be stewards of nature. We are not called upon to

passively observe, but to participate and protect. This may seem as though I am advocating for the protection of *M. genitalium*, but I emphasize my point again that humans are part of nature. That means we have a duty to participate with other organisms while protecting ourselves from their threat. This calling of stewardship and responsibility demands that we recognize our own place within nature and the impact we can have on it, but also the impact it can have on us.

When we talk about the evolution of organisms, we are talking about small changes in a species over a large portion of time. We tend to think of ourselves as separate from the change around us. However, our own body is modifying which antibodies it makes to be more effective at protecting itself from its invaders. While the *M. genitalium* microbe was using its genetic abilities to create variant versions of itself to evade the immune system, our immune system was learning which regions weren't variable and was targeting those constant regions. Part of what it means to be human is to recognize that humans are animals that do not exist outside of the rest of the world. We are our bodies, and we are constantly being forced to interact with other organisms. It is our body's responsibility to respond to the world around it, and we have the obligation to not separate our physical body from our humanity. It is how the rest of the world interacts with us and how we interact with the rest of the world.

REFERENCES TO PICTURES USED IN APPENDIX AND PRESENTATION

Brent Cornell. BioNinja. (n.d.). https://ib.bioninja.com.au/standard-level/topic-6-human-physiology/63defence-against-infectio/lymphocytes.html.

Europe, P. D. B. in. (n.d.). *EMD-3756*. EBI. https://www.ebi.ac.uk/pdbe/entry/emdb/EMD 3756/analysis.

McGowin, C. L., & Totten, P. A. (2017). The Unique Microbiology and Molecular Pathogenesis of Mycoplasma genitalium. *The Journal of Infectious Diseases*, *216*(suppl_2). https://doi.org/10.1093/infdis/jix172

Wikimedia Foundation. (2021, May 11). *Surface plasmon resonance*. Wikipedia. https://en.wikipedia.org/wiki/Surface_plasmon_resonance#/media/File:Surface_Plasmon_Resonance_(SPR).jpg.

Wong, C. (2019, February 18). *Mycoplasma genitalium: Challenges in Diagnosis and Treatment*. Infectious Disease Advisor. https://www.infectiousdiseaseadvisor.com/home/topics/sexually-transmitted-diseases/mycoplasma-genitalium-challenges-in-diagnosis-and-treatment/.

Wood, G. E., Iverson-Cabral, S. L., Gillespie, C. W., Lowens, M. S., Manhart, L. E., & Totten, P. A. (2020). Sequence variation and immunogenicity of the Mycoplasma genitalium MgpB and MgpC adherence proteins during persistent infection of men with non-gonococcal urethritis. *PLOS ONE*, *15*(10). https://doi.org/10.1371/journal.pone.0240626