Anti-Psl Targeting of *Pseudomonas aeruginosa* Biofilms for Neutrophil-Mediated Disruption

Valerie A. Ray[1], Preston J. Hill[1], C. Kendall Stover[2], Sashwati Roy[3,4], Chandan K. Sen[3,4], Li Yu[5], Daniel J. Wozniak[1], Antonio DiGiandomenico[2*]

[1] Center for Microbial Interface Biology, Departments of Microbial Infection and Immunity, Microbiology, Ohio State University, Columbus, OH 43210, USA.

[2] Department of Infectious Diseases, MedImmune, LLC, Gaithersburg, MD 20878, USA.

[3] Comprehensive Wound Center, Davis Heart and Lung Research Institute, Centers for Regenerative Medicine and Cell Based Therapies, Ohio State University Medical Center, Columbus, OH 43210, USA.

[4] Department of Surgery, College of Veterinary Medicine, Ohio State University, Columbus, OH 43210, USA.

[5] Translational Sciences, MedImmune, LLC, Gaithersburg, MD 20878, USA.

Supplemental Information

Supplementary Fig. S1. Structure of Psl and synthetic Psl oligosaccharides. (A) Cell-free Psl is a pentasaccharide repeat unit. (B-E) Synthetic Psl oligosaccharides used to evaluate binding of anti-Psl mAbs which recognized unique epitopes (class I, II and III) [1]. (B) Psl tetrasaccharide – bound by the class II mAb. (C) Psl pentasaccharide – bound by the class II mAb, (D) Psl hexasaccharide – bound by class II and class III mAbs. (E) Psl di-pentasaccharide – bound by the class II mAb and weakly by the class III mAb.

Supplementary Fig. S2. Individual anti-Psl mAbs stain PAO1 biofilms. Flow grown biofilms of PAO1 stained with either (A) class I (green), (B) class II (orange), or (C) class III (red) anti-Psl mAbs, imaged via CLSM at 10x magnification, and processed via IMARIS. Scale bars represent 150 µm.

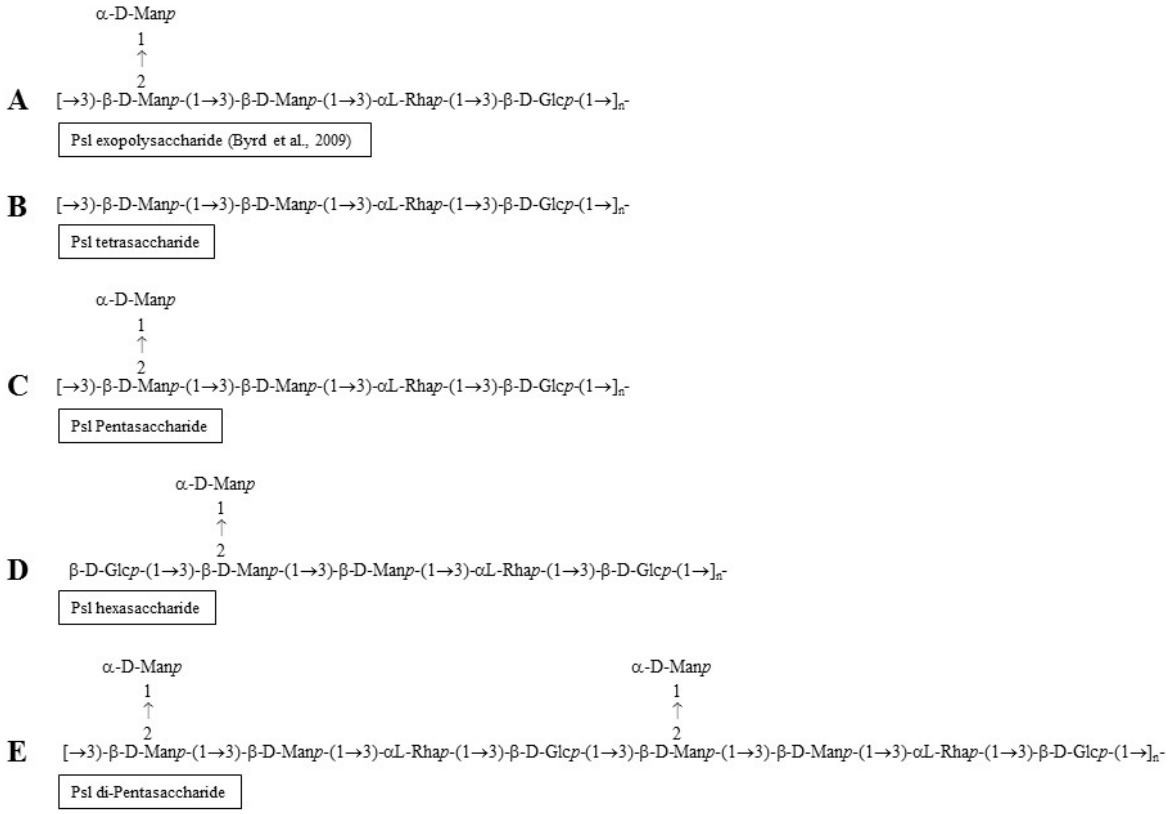
Supplementary Fig. S3. Thermally injured porcine tissue does not react with an isotype control IgG mAb. *P. aeruginosa* infected skin from thermally injured pigs was imaged via CLSM at 10x magnification. (A) DIC image (same as Fig. 3), (B) DAPI staining, and (C) control IgG mAb staining. (D-F) The wound bed is below the white dotted line and white dashed line represents below the wound bed. Scale bars represent 100 µm.

Supplementary Figure S4. Anti-Psl mAbs elicit a phagocytic burst response from human neutrophils. Planktonic PAO1 was incubated with neutrophils and PMA (positive control), non-opsonized or IgG isotype control antibody (negative controls), or

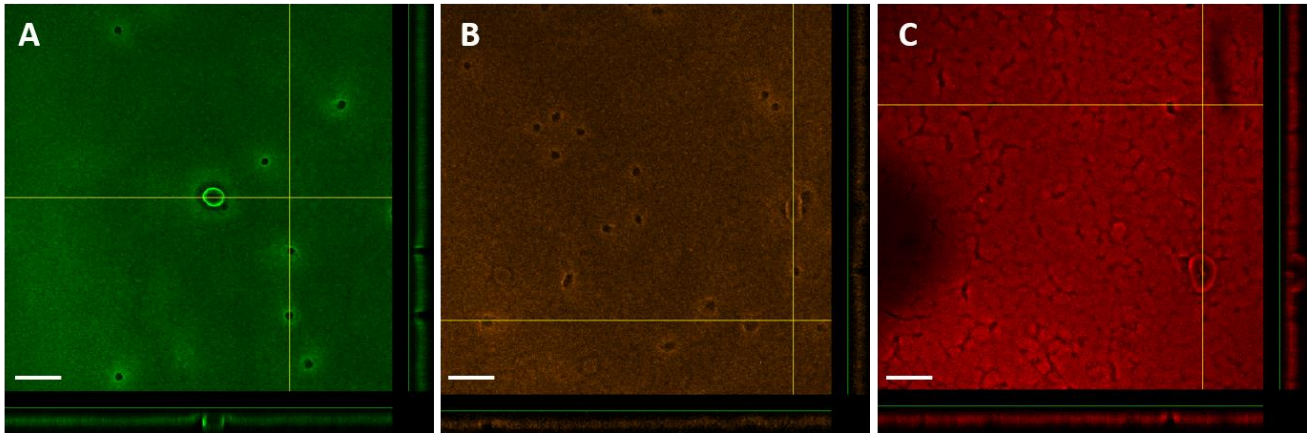
opsonized with human serum, individual anti-Psl mAbs, or a combination of all three mAbs.

Supplementary Movie S1. Anti-Psl mAbs differentially stain the biofilm. IMARIS generated movie from image shown in Figure 2B with removal of layers (class I – green, class II – yellow/orange, and class III – red) and rotation to demonstrate differential staining.

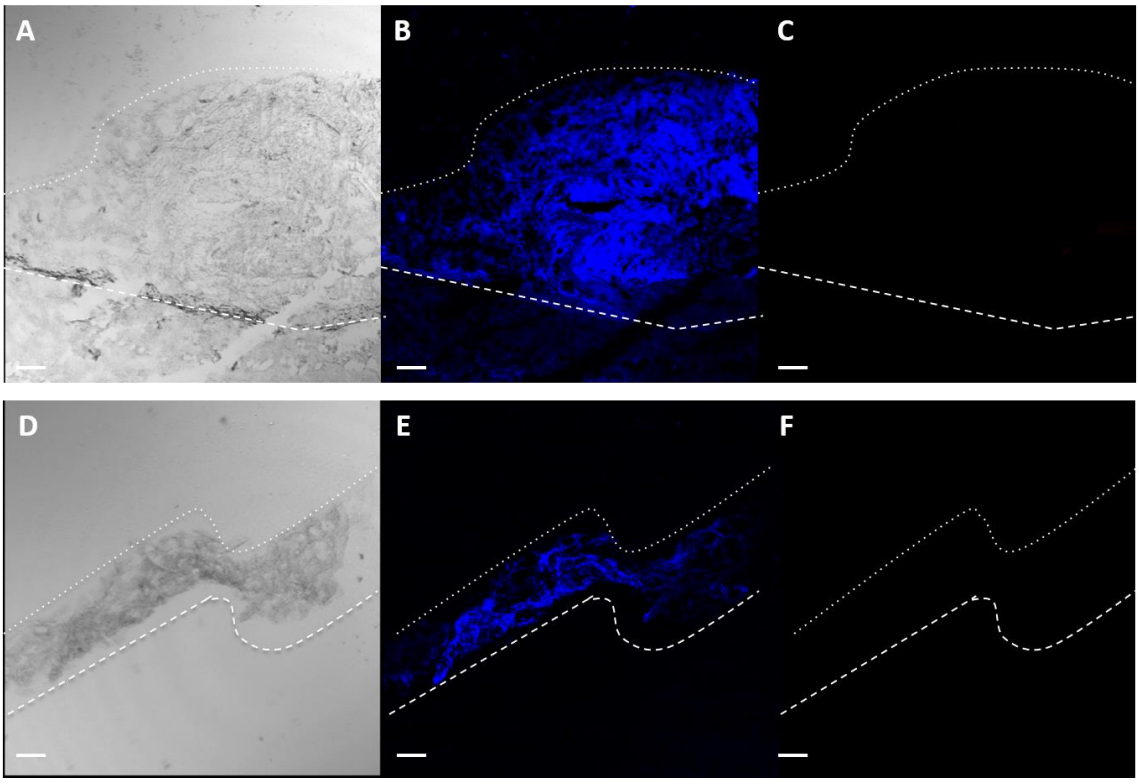
Supplementary Fig. S1



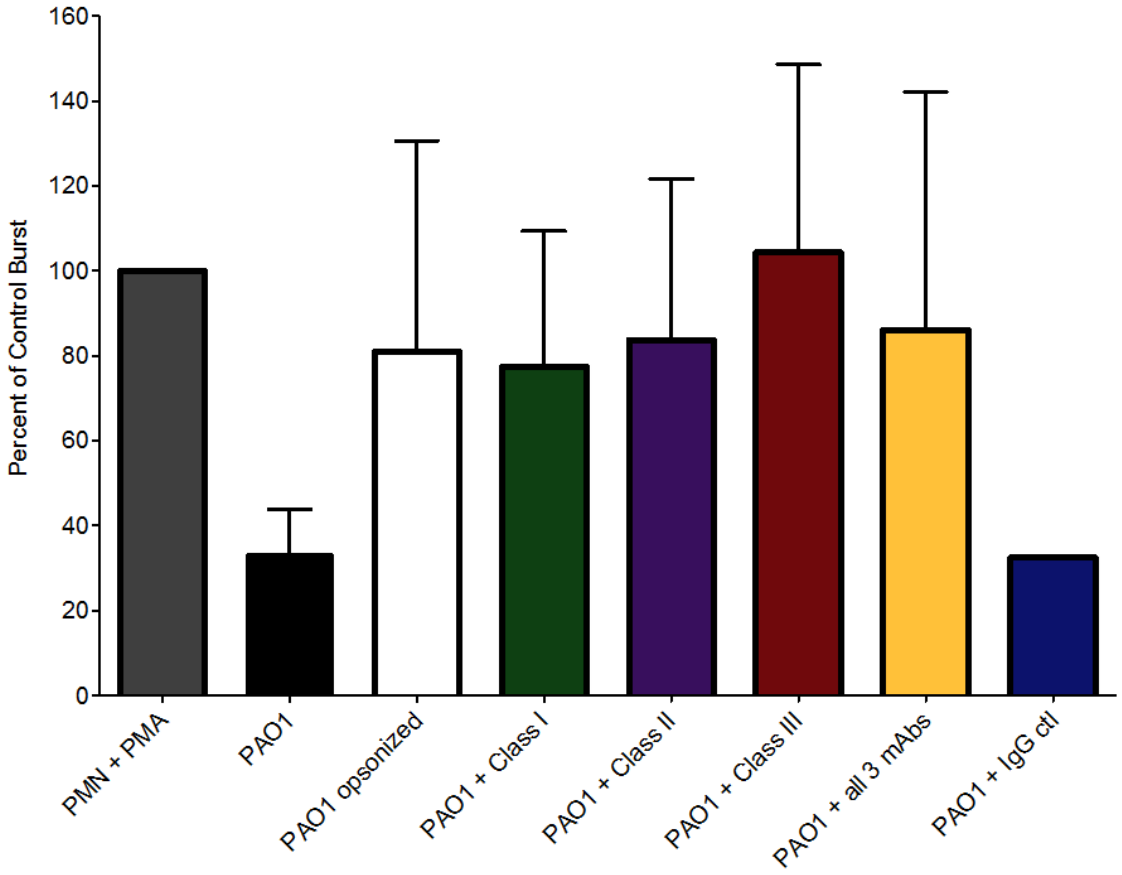
Supplementary Fig. 2



Supplementary Fig. S3



Supplementary Fig. S4



Supplementary TABLE S1. Raw data from biofilm attachment assays.

Experiment 1

Condition	O.D. A_{540}	Condition	O.D. A_{540}
Δ*psl* #1	0.033	PAO1 #1	0.121
Δ*psl* #2	0.038	PAO1 #2	0.158
Δ*psl* #3	0.050	PAO1 #3	0.128
IgG Control #1	0.111	Class I #1	0.037
IgG Control #2	0.150	Class I #2	0.056
IgG Control #3	0.135	Class I #3	0.070
Class II #1	0.070	Class III #1	0.080
Class II #2	0.126	Class III #2	0.081
Class II #3	0.141	Class III #3	0.059
All 3 mAbs #1	0.102		
All 3 mAbs #2	0.121		
All 3 mAbs #3	0.106		

Experiment 2

Condition	O.D. A_{540}	Condition	O.D. A_{540}
Δ*psl* #1	0.049	PAO1 #1	0.146
Δ*psl* #2	0.060	PAO1 #2	0.155
Δ*psl* #3	0.057	PAO1 #3	0.166
IgG Control #1	0.143	Class I #1	0.066
IgG Control #2	0.160	Class I #2	0.129
IgG Control #3	0.150	Class I #3	0.116
Class II #1	0.128	Class III #1	0.162
Class II #2	0.112	Class III #2	0.088
Class II #3	0.137	Class III #3	0.071
All 3 mAbs #1	0.122		
All 3 mAbs #2	0.134		
All 3 mAbs #3	0.105		

Experiment 3

Condition	O.D. A_{540}	Condition	O.D. A_{540}
Δ*psl* #1	0.031	PAO1 #1	0.155
Δ*psl* #2	0.059	PAO1 #2	0.174
Δ*psl* #3	0.061	PAO1 #3	0.168
IgG Control #1	0.164	Class I #1	0.080
IgG Control #2	0.173	Class I #2	0.122
IgG Control #3	0.159	Class I #3	0.047

Class II #1	0.293		Class III #1	0.155
Class II #2	0.108		Class III #2	0.107
Class II #3	0.115		Class III #3	0.102
All 3 mAbs #1	0.104			
All 3 mAbs #2	0.092			
All 3 mAbs #3	0.104			

Experiment 4

Condition	O.D. A_{540}	Condition	O.D. A_{540}
Δ*psl* #1	0.067	PAO1 #1	0.129
Δ*psl* #2	0.067	PAO1 #2	0.152
Δ*psl* #3	0.048	PAO1 #3	0.147
IgG Control #1	0.105	Class I #1	0.103
IgG Control #2	0.125	Class I #2	0.117
IgG Control #3	0.155	Class I #3	0.127
Class II #1	0.111	Class III #1	0.110
Class II #2	0.122	Class III #2	0.112
Class II #3	0.133	Class III #3	0.133
All 3 mAbs #1	0.106		
All 3 mAbs #2	0.121		
All 3 mAbs #3	0.120		

Supplementary TABLE S2. Raw data from biofilm aggregation assays.

Experiment 1

Condition	O.D. A_{600}	O.D.A_{490}	Aggregation Index (A_{490}/A_{600})
Δ*psl* #1	0.091	1.134	12.462
Δ*psl* #2	0.093	1.122	12.065
Δ*psl* #3	0.093	1.142	12.280
WFPA 801 #1	0.072	1.196	16.611
WFPA 801 #2	0.071	1.114	15.690
WFPA 801 #3	0.074	1.160	15.676
WFPA 801 + ara #1	0.040	0.940	23.500
WFPA 801 + ara #2	0.043	1.000	23.256
WFPA 801 + ara #3	0.045	1.044	23.200
IgG Control #1	0.044	1.000	22.727
IgG Control #2	0.042	0.982	23.381
IgG Control #3	0.044	1.018	23.136
Class I #1	0.054	1.068	19.778
Class I #2	0.053	1.036	19.547
Class I #3	0.056	1.092	19.500
Class II #1	0.053	1.066	20.113
Class II #2	0.055	1.058	19.236
Class II #3	0.048	1.038	21.625
Class III #1	0.049	1.100	22.449
Class III #2	0.048	1.068	22.208
Class III #3	0.050	1.114	22.280
All 3 mAbs #1	0.066	1.210	18.333
All 3 mAbs #2	0.059	1.068	18.102
All 3 mAbs #3	0.060	1.136	18.933

Experiment 2

Condition	O.D. A_{600}	O.D.A_{490}	Aggregation Index (A_{490}/A_{600})
Δ*psl* #1	0.090	1.016	11.289
Δ*psl* #2	0.094	1.111	11.819
Δ*psl* #3	0.103	1.158	11.243
WFPA 801 #1	0.071	1.098	15.465
WFPA 801 #2	0.074	1.128	15.243
WFPA 801 #3	0.080	1.210	15.125
WFPA 801 + ara #1	0.053	1.106	20.868
WFPA 801 + ara #2	0.055	1.130	20.545
WFPA 801 + ara #3	0.059	1.186	20.102

IgG Control #1	0.056	1.112	19.857
IgG Control #2	0.059	1.100	18.644
IgG Control #3	0.057	1.168	20.491
Class I #1	0.057	1.054	18.491
Class I #2	0.057	1.096	19.228
Class I #3	0.059	1.102	18.678
Class II #1	0.056	1.044	18.643
Class II #2	0.058	1.138	19.621
Class II #3	0.058	1.162	20.034
Class III #1	0.051	1.038	20.353
Class III #2	0.058	1.344	23.172
Class III #3	0.057	1.122	19.684
All 3 mAbs #1	0.062	0.994	16.032
All 3 mAbs #2	0.063	1.008	16.000
All 3 mAbs #3	0.066	1.060	16.061

Experiment 3

Condition	O.D. A_{600}	O.D.A_{490}	Aggregation Index (A_{490}/A_{600})
Δ*psl* #1	0.120	0.849	7.075
Δ*psl* #2	0.110	0.877	7.973
Δ*psl* #3	0.122	0.816	6.689
WFPA 801 #1	0.107	1.076	10.056
WFPA 801 #2	0.112	1.096	9.786
WFPA 801 #3	0.126	1.124	8.921
WFPA 801 + ara #1	0.084	1.050	12.500
WFPA 801 + ara #2	0.086	1.146	13.326
WFPA 801 + ara #3	0.090	1.158	12.867
IgG Control #1	0.088	0.906	10.295
IgG Control #2	0.089	0.988	11.101
IgG Control #3	0.081	0.892	11.012
Class I #1	0.097	1.064	10.969
Class I #2	0.089	1.030	11.573
Class I #3	0.096	1.132	11.792
Class II #1	0.082	0.986	12.024
Class II #2	0.085	1.014	11.929
Class II #3	0.090	1.024	11.378
Class III #1	0.086	1.032	12.000
Class III #2	0.085	1.028	12.094
Class III #3	0.084	1.018	12.119
All 3 mAbs #1	0.081	0.992	12.247

All 3 mAbs #2	0.082	0.980	11.951
All 3 mAbs #3	0.087	1.032	11.862

Experiment 4

Condition	O.D. A_{600}	O.D.A_{490}	Aggregation Index (A_{490}/A_{600})
Δ*psl* #1	0.198	0.973	4.914
Δ*psl* #2	0.209	0.903	4.321
Δ*psl* #3	0.208	0.991	4.764
WFPA 801 #1	0.213	1.078	5.061
WFPA 801 #2	0.215	1.184	5.507
WFPA 801 #3	0.249	1.166	4.683
WFPA 801 + ara #1	0.104	1.002	9.635
WFPA 801 + ara #2	0.106	0.980	9.245
WFPA 801 + ara #3	0.098	1.042	10.633
IgG Control #1	0.109	1.022	9.376
IgG Control #2	0.101	1.018	10.079
IgG Control #3	0.105	1.058	10.076
Class I #1	0.132	0.894	6.773
Class I #2	0.124	0.838	6.758
Class I #3	0.134	0.928	6.925
Class II #1	0.132	0.950	7.197
Class II #2	0.142	0.966	6.803
Class II #3	0.138	1.114	8.072
Class III #1	0.140	1.098	7.843
Class III #2	0.133	1.050	7.895
Class III #3	0.130	1.062	8.169
All 3 mAbs #1	0.153	1.006	6.575
All 3 mAbs #2	0.161	1.106	6.870
All 3 mAbs #3	0.164	1.080	6.585

References

1 Li, H. *et al.* Epitope mapping of monoclonal antibodies using synthetic oligosaccharides uncovers novel aspects of immune recognition of the Psl exopolysaccharide of Pseudomonas aeruginosa. *Chemistry* **19**, 17425-17431, doi:10.1002/chem.201302916 (2013).