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Antibody-Mediated Opsonophagocytosis of *Yersinia pestis* Induces Potentially Anti-Protective IL-10 Secretion

[1]Yuli Talyansky; [1]Christopher P. Klimko; [1]Melissa Hunter; [1]Jennifer Shoe; [1]Jennifer Dankmeyer; [1]Sergei S. Biryukov; [2]Marcellene A. Gates-Hollingsworth; [2]Derrick Hau; [2]David P. AuCoin; and [1]Christopher K. Cote
[1]Bacteriology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland. [2]University of Nevada - Reno, Reno, Nevada.

1. Introduction

- *Yersinia pestis*, the causative agent of bubonic and pneumonic plague, remains a significant public health and biothreat risk with few targeted treatment and immunization options.
- Antibodies targeting the F1 capsule and LcrV antigen have been developed but their immunomodulatory properties have not been well described.
- LcrV is part of the Type III secretion system and can independently induce an immunosuppressive IL-10 response.
- Certain IgG isotypes preferentially bind Fc receptors carrying ITAM- or ITIM-containing motifs, potentially polarizing immune response in addition to inducing opsonophagocytosis.

2. Materials and Methods

- *Y. pestis* CO92 (*pgm-*/pPst-) were grown overnight at 28 °C, transitioned to 37 °C 2 hours prior to co-incubation of bacteria and antibodies for 1 h. RAW264.7 cells were subsequently infected with antibody-bound bacteria at an MOI of 10, incubated for 2 hours, and transitioned to gentamicin-containing media to kill extracellular bacteria. Where appropriate, RAW cells were incubated with 100 µg/mL of anti-Fc antibodies for 1 h prior to infection. Supernatants were collected, and RAW cells were lysed with 0.1% Triton-X for subsequent plating of intracellular bacteria. Supernatants were analyzed using a 36-plex Luminex mouse cytokine panel on a MagPix instrument.
- For *in vivo* infection, 8-week female BALB/c mice were infected via whole-body aerosol with approximately 1.0 LD_{50} ($6.8x10^4$ CFUs) of wild-type CO92. Animals received 25 µg of anti-LcrV mAb 7.3 or a PBS control and/or 200 µg of anti-mouse IL-10 antibody or rat IgG control intraperitoneally 18 hours prior to infection.

3. Results

	7.3 (IgG1)	mIgG	2B2 (IgG2a)
IL-10*	**3.59**	0.56	0.25
LIF*	**1.95**	0.41	0.29
IL-9	**1.87**	1.87	1.55
M-CSF*	**1.84**	0.57	0.58
IL-1α*	**1.19**	0.76	0.94
GM-CSF*	**1.17**	0.49	0.35
IL-2*	**1.06**	0.59	0.54
IL-6*	**1.03**	0.34	0.23
IFN-γ*	**1.01**	0.56	0.44
MCP-1	1.00	1.00	1.00
MIP-1b	1.00	1.00	1.49
MIP-1a	1.00	1.00	1.00
MIP-2	1.00	1.00	1.00
RANTES	1.00	1.00	1.00
MCP-3*	**0.99**	0.37	0.34
IL-18*	**0.95**	0.53	0.42
IL-12p70*	**0.94**	0.53	0.43
IL-28	0.94	0.19	0.69
Eotaxin*	**0.93**	0.67	0.62
IL-23	0.92	0.85	1.06
IL-3	0.90	0.84	0.94
IL-15	0.87	0.59	0.67
IL-5*	**0.86**	0.52	0.50
IL-13*	**0.83**	0.45	0.32
IL-4	0.78	0.55	0.61
Gro-α	0.73	0.86	0.72
ENA-78	0.71	0.74	0.77
IL-17A	0.69	0.70	0.85
IFN-α	0.66	0.66	0.66
G-CSF	0.58	0.44	0.36
TNF-α	0.48	0.42	0.37
IL-22	0.47	0.71	0.93
IL-27	0.38	0.44	0.38
IP-10	0.27	0.47	0.40
IL-1β*	**0.17**	0.84	0.93

Fig 1: Cytokine Expression of Anti-LcrV Antibodies. RAW264.7 cells were infected with *Yp* CO92(*pgm-*/pPst-) at an MOI of 10:1. Supernatants were collected at 24 hours and cytokines measured via Luminex. * = p < 0.05 via Mann-Whitney test, vs. mIgG.

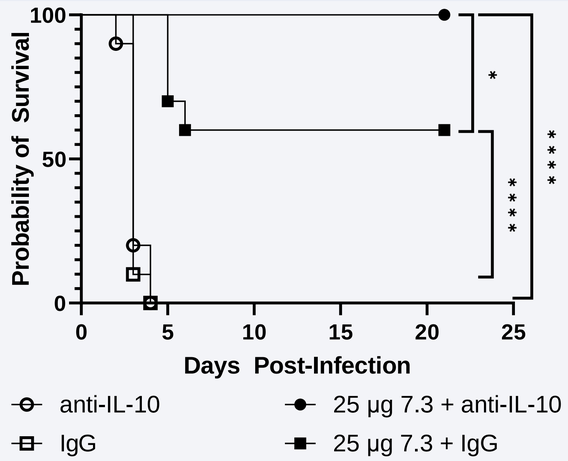

Fig 2: *In Vivo* Survival. 8-week female BALB/c mice were infected via whole-body aerosol with 1.0 LD_{50} of wild-type CO92 with or without 25 µg of 7.3 antibody and/or an anti-mouse IL-10 neutralizing rat antibody or rat IgG control. * = p < 0.05, **** = p < 0.001 via Mantel-Cox test.

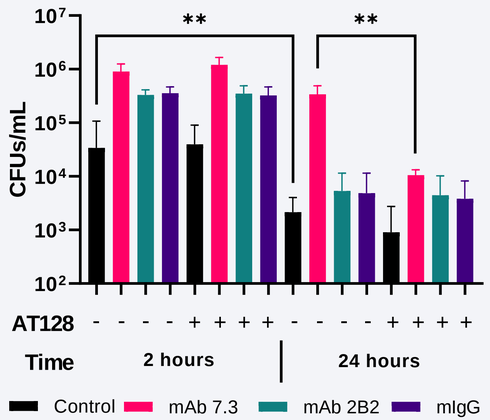

Fig 3: Invasion Assay. RAW264.7 cells were pre-treated with anti-FcγRIIb antibody AT128, co-incubated with anti-LcrV antibody-bound bacteria, and internalized bacteria were enumerated. ** = p < 0.01 via Mann-Whitney test.

G-CSF	1.02	1.28	1.11	1.14	1.04	1.29
IL-10	1.67	2.13	2.10	0.29	0.20*	0.19
IL-3	2.06	1.99	1.65	0.63	0.28	0.21
LIF	1.25	1.41	1.32	0.44	0.34	0.42
IL-1β	0.52	0.58	0.48*	0.21	0.19	0.17
IL-2	1.55	1.38	1.41	0.55	0.40	0.41*
M-CSF	3.07	3.05	3.82	1.83	1.56	1.65
IP-10	0.65	0.88	0.71	0.88	0.84	0.86
IL-4	0.92	1.07	0.96	0.82	0.64	0.70
IL-5	1.09	1.21	0.99	0.83	0.56	0.73
IL-6	1.88	0.69	5.04	0.27	0.21	0.24
IFN-α	1.48	2.79	1.48	2.79	3.28	3.28
IL-22	1.02	0.94	1.06	0.48	0.44	0.39
IL-9	1.13	1.24	1.36	5.02	4.81	4.34
IL-13	1.00	1.04	1.08	0.92	0.68	0.78
IL-27	0.83	1.00	1.05	0.24	0.26	0.26
IL-23	0.90	1.05	1.05	0.93	0.70	0.75
IFN-γ	0.99	1.03	1.02	0.74	0.57	0.55*
IL-12p70	1.49	1.40	1.27	0.76	0.46	0.48
GM-CSF	0.61	0.72	0.64	0.27	0.23	0.29
GRO-α	0.45	0.60	0.51	0.31	0.29	0.35
TNF-α	1.07	1.29	1.01	1.13	0.91	1.15
MCP-3	2.23	1.86	1.55	0.76	0.60	0.73
MCP-1	0.41	0.41	2.40	0.76	0.43	0.48
IL-17A	1.13	1.24	1.13	0.91	0.65	0.70
IL-15	2.07	2.36	2.51	0.72	0.49*	0.16*
IL-1α	1.58	1.73	1.58	0.33	0.23	0.21
ENA-78	1.43	1.23	1.33	0.94	0.81	0.93
Eotaxin	1.14	1.20	1.07*	0.78	0.61*	0.75
IL-28	0.97	1.27	1.02	0.60	0.45	0.42
IL-18	6.38	6.96	5.25	3.33	2.74	1.94
mAb	7.3	7.3	7.3	2B2	2B2	2B2
FcγRIIb		↓	↑		↓	↑

Fig 4: Cytokine Expression with FcγRIIb Blockade. RAW264.7 cells were pre-treated with inhibitory anti-FcγRIIb antibody AT128, or activatory AT130-2, co-incubated with anti-LcrV antibody-bound bacteria, and supernatants measured via Luminex. Expressed as fold change vs. bacteria alone. * = p < 0.05 via Mann-Whitney test vs. anti-V mAb alone.

4. Discussion

- Opsonophagocytosis of *Yersinia pestis* with anti-LcrV antibodies may differentially induce cytokines based on isotype, with IgG1 antibodies upregulating immunosuppressive cytokines such as IL-10 and M-CSF. Partial rescue of infected mice with IgG1 antibodies can be boosted by the addition of IL-10-neutralizing antibodies.
- Neutralizing antibodies against the ITIM-bearing FcγRIIb receptor may blunt IgG1-mediated immunosuppression and improve intracellular killing in RAW264.7 macrophage-like cells.
- Future development of candidate vaccines and therapeutic antibodies should interrogate the role of isotypes and Fc-mediated polarization on host response to optimize therapeutic value.

Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 2011. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

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POINTS OF CONTACT
Yuli Talyansky
yuli.talyansky.ctr@health.mil
Christopher Cote
christopher.k.cote.civ@health.mil