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1000. Serotype 3 pneumococci evade activation of the classical complement pathway

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Session: P-56. Microbial Pathogenesis

Background. Complement classical pathway (CCP) activation is the major mechanism leading to opsonophagocytic pneumococcal killing. Following immunization with 13-valent pneumococcal conjugate vaccine (PCV13), opsonophagocytic titers are lowest against serotype 3 among the 13 vaccine serotypes. Post licensure surveillance indicated early declines in serotype 3 invasive pneumococcal disease (IPD) were not sustained over time

C3 and C4 deposition on Clade I and Clade II serotype 3 Nasopharyngeal Isolates from Children in Boston

NP-CLADE I

Strain	Complement only		Monoclonal anti-capsular IgG 1% [10ug/ml]		Monoclonal anti-capsular IgG 3% [30ug/ml]		RPS3A		RPS3A + anti-rabbit IgM	
	C3#	C4#	C3#	C4#	C3#	C4#	C3#	C4#	C3#	C4#
1445	92.1	2.8	95.2	12.6	96.2	29.8	99.6	99	99.5	16.3
2414	97.8	5.9	98.1	20.4	99	47	99.7	99	99.2	32.6
6910	92.8	13.1	98.1	7.5	95.4	23.7	99.3	98.6	99.2	12.5
7920	98.4	1.3	98.9	6.4	99.4	19	99.7	98.6	99.4	3.7
1457	92.4	4.9	96	9.8			97.5	98.6	99.4	9.9
7970	91.1	5.7	96.2	16.5			98.8	95.8	98.8	51.9
1344	26.8	3.7	69.7	7.2			99.7	97.6	96.4	11.4
2242	96	3.4	98	12.5			99.8	99.1	99.7	12.5
MEDIAN	**92.6**	**4.3**	**97.1**	**11.2**	**97.6**	**26.7**	**99.6**	**98.6**	**99.3**	**12.5**

proportion of pneumococci cells binding C3 or C4
Rabbit polyclonal serotype 3 antisera [RPS3A]

NP – Clade II

Strain	Complement only		Monoclonal anti-capsular IgG 1% [10ug/ml]		Monoclonal anti-capsular IgG 3% [30ug/ml]		RPS3A		RPS3A + anti-rabbit IgM	
	C3#	C4#	C3#	C4#	C3#	C4#	C3#	C4#	C3#	C4#
1108	19.6	10.3	60.6	12	72.5	22.4	99.2	91.2	94.5	19.2
1961	16.4	3.2	64.6	11.2	73.3	22.3	99.4	73.7	97.1	14.6
2219	31.3	3.9	75.9	8.43	86.1	27.1	99.6	96	99.5	31.8
3035	35.3	3.3	85.8	6.8	88.3	19.3	99.6	95.9	99.6	9.8
3777	58.3	14.5	65.6	17.3			99.5	97	98.9	39.6
4346	36.7	4.6	87.4	7.2			99.4	99.1	99.4	21.7
4715	86.9	2.8	74.1	10.7			99.8	96.2	99.1	15
5685	24.9	2.3	63.9	8.8			99.4	96.1	95	19.9
MEDIAN	**33.3**	**3.6**	**70**	**9.7**	**79.7**	**22.3**	**99.4**	**96**	**99**	**19.5**

proportion of pneumococci cells binding C3 or C4
Rabbit polyclonal serotype 3 antisera [RPS3A]

Methods. Using flow cytometry, we measured C3 and C4 deposition on serotype 3 strains from children with IPD or nasopharyngeal [NP] carriage, and analyzed by clade. C4 deposition is an indicator of CCP, while C3 deposition is common to all complement pathways. We measured C3/C4 deposition on serotype 3 pneumococcal strains incubated with antibody depleted complement alone or with complement and the following antibodies: mouse monoclonal anti-capsular IgG or IgM, rabbit polyclonal serotype 3 antisera (IgG + IgM) [RPS3A] and RPS3A combined with anti-rabbit IgM, which blocks IgM function, leaving only polyclonal IgG

Results. Serotype 3 strains demonstrated high variability in C3 binding when incubated with complement alone. RPS3A (containing both IgM+IgG) and monoclonal IgM activated CCP in all strains. Anti- serotype 3 monoclonal IgG and polyclonal IgG demonstrated absent or limited CCP activation; but activated alternative pathway in some strains. When analyzing complement deposition by clade, a lower proportion of clade II NP serotype 3 strains bound C3 when incubated with complement or monoclonal IgG, compared to clade Ia NP strains. Differences between clade Ia and II IPD strains were not apparent.

Conclusion. Serotype 3 strains did not demonstrate activation of the CCP in the presence IgG and varied in C3 deposition. Pneumococcal strains that evade CCP activation may be less sensitive to opsonophagocytosis. Our findings suggest a mechanism by which serotype 3 carriage and disease may persist despite immunization with conjugate vaccine containing serotype 3 polysaccharide.

Disclosures. **Rotem Lapidot, MD MSCI**, **Pfizer** (Consultant, Grant/Research Support, Advisor or Review Panel member) **Mario Ramirez, PhD**, **GlaxoSmithKline** (Advisor or Review Panel member)**Merck Sharp & Dohme** (Advisor or Review Panel member)**Pfizer** (Speaker's Bureau) **Ingrid L. Scully, PhD**, **Pfizer** (Employee, Shareholder) **Bradford D. Gessner, MD, MPH**, **Pfizer Inc.** (Employee) **stephen pelton, MD**, **Merck Vaccines** (Advisor or Review Panel member, Research Grant or Support)**Pfizer, Inc.** (Consultant, Advisor or Review Panel member, Research Grant or Support)**Sanofi pasteur** (Advisor or Review Panel member, Research Grant or Support, DSMB)**Seqirus** (Consultant)

1001. Chronic Colonization with Toxigenic *Clostridioides difficile* Strains Drives Colonic Tumorigenesis in Mice

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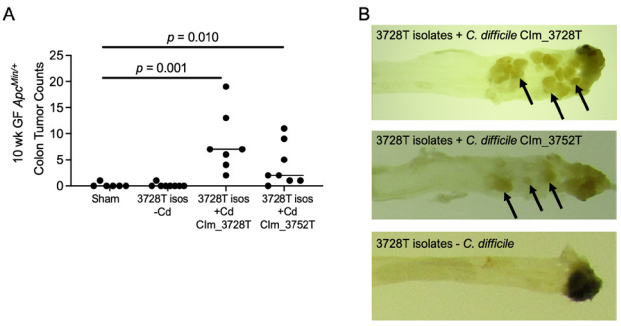
Session: P-56. Microbial Pathogenesis

Background. Long-term effects of chronic and/or recurrent *C. difficile* infections (CDI) are not well understood, and any potential role of CDI in colorectal cancer (CRC) risk is presently unknown. While pursuing efforts to identify novel procarcinogenic microbes, we identified two mucosal slurries from CRC patients (3728T and 3752T) that were tumorigenic in germ-free (GF) *ApcMin*/+ mice. Surprisingly, both of these CRC patient slurries were positive for *C. difficile* by 16S rRNA amplicon sequencing. Given the ability of other chronic infections to promote tumorigenesis (e.g., *H. pylori*), we hypothesized that chronic colonization with *C. difficile* could promote tumorigenesis in the colon.

Methods. A consortium of 30 bacterial isolates including a toxigenic *tcdA*+ *tcdB*+ *C. difficile* strain (CIm_3728T) was cultured from GF *ApcMin*/+ mice gavaged with the 3728T slurry. This consortium was gavaged into additional GF *ApcMin*/+ mice with or without *C. difficile* strains CIm_3728T, CIm_3752T (isolated from mice gavaged with the 3752T slurry), or isogenic *tcdA*/*tcdB* mutants of the M7404 R027 strain. Single cell RNA sequencing (scRNAseq), high dimensional (HD) flow cytometry, and fluorescence *in situ* hybridization (FISH) with EUB338 and Cd198 probes were performed on distal colons from mice gavaged with either complex CRC slurries or the 3728T isolates with CIm_3728T.

Results. *C. difficile* strains drove tumorigenesis of the 3728T isolate mixture (**Fig. 1A,B**). Tumorigenesis was associated with early procarcinogenic signaling and spatial changes including induction of Wnt signaling in colonic epithelial progenitor cells by scRNAseq, IL-17 induction in immune cells by HD flow cytometry, and bacterial biofilm invasion deep into epithelial crypts by FISH. Tumorigenesis correlated with chronic colonization with toxigenic strains of *C. difficile* and was toxin-dependent, as toxin mutant strains (M7404 *tcdA-tcdB*-) did not induce tumors.

Figure 1. C. difficile strains from CRC patients induce distal colonic tumorigenesis in germ-free (GF) ApcMin/+ mice.



A consortium of 30 bacteria, including C. difficile, were isolated from mice gavaged with the 3728T human CRC mucosal slurry. These isolates were then gavaged into additional GF ApcMin/+ mice, with or without C. difficile isolates from mice gavaged with the 3728T slurry or 3752T slurry. (A) Colonic tumor numbers in GF ApcMin/+ mice at 10 wk p.i. demonstrate that C. difficile (Cd) drives the tumorigenesis of this 30-member bacterial consortium. (B) Gross tumors can be observed in the colon of a representative mouse gavaged with the 3728T isolates with the CIm_3728T (top) or CIm_3752T (middle) strain of C. difficile but not in a mouse gavaged with the isolates lacking C. difficile (bottom).

Conclusion. Toxigenic *C. difficile* strains isolated from human CRC mucosal slurries were pro-carcinogenic in mice, suggesting that *C. difficile* is a potential driver of CRC. Given the public health burden of *C. difficile*, further studies are warranted to determine whether *C. difficile* infections (initial, recurrent, and chronic asymptomatic) increase CRC risk in patients.

Disclosures. **Jada Domingue, PhD**, **AstraZeneca** (Employee) **James White, PhD**, **Personal Genome Diagnostics** (Consultant) **Patricia J. Simner, PhD**, **Accelerate Diagnostics** (Grant/Research Support)**Affinity Biosensors** (Grant/Research

Support)**BD Diagnostics** (Consultant, Grant/Research Support)**GeneCapture** (Consultant)**OpGen, Inc** (Consultant, Grant/Research Support)**Shionogi, Inc** (Consultant) **Karen C. Carroll, MD**, **MeMed** (Scientific Research Study Investigator)**Meridian Diagnostics, Inc.** (Grant/Research Support)**Pattern Diagnostics** (Advisor or Review Panel member)**Scanogen, Inc.** (Advisor or Review Panel member) **Karen C. Carroll, MD**, Pattern Diagnostics, Inc. (Individual(s) Involved: Self): Grant/Research Support; Scanogen, Inc. (Individual(s) Involved: Self): Consultant **Cynthia L. Sears, MD**, **Bristol Myers Squibb** (Grant/Research Support)**Ferring** (Advisor or Review Panel member)**Janssen** (Grant/Research Support)

1002. Activation of the *Enterococcus faecalis* Cell Envelope Stress Response through the Novel MadRS System Is Associated With Increased Size of Cardiac Microlesions

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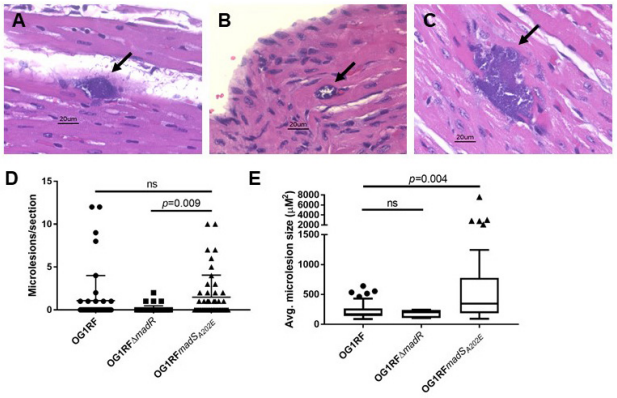
Session: P-56. Microbial Pathogenesis

Background. Enterococci are opportunistic pathogens that can present a therapeutic challenge due to the acquisition of antibiotic resistance. Our previous work has shown the MadRS stress response system plays an important role in defending the enterococcal membrane against daptomycin and antimicrobial peptides (AMP) made by the innate immune system. Strains lacking the MadR response regulator show increased susceptibility to the cathelicidn LL-37 in vitro. A change from alanine to glutamate in the sensor kinase MadS (*madSA202E*) leads to activation of the system and impaired killing by AMPs. In this study, we evaluated the impact of MadRS function in vivo using a mouse peritonitis model of *E. faecalis* (*Efs*) infection.

Methods. A laboratory strain *Efs* OG1RF and two derivatives, OG1RFΔ*madR* and OG1RF*madSA202E* were included. Six mice per strain were inoculated via intraperitoneal injection of ~5x10^8 CFU/mL of bacteria in 50% sterile rat fecal extract, and followed for 96 hours post infection. Difference in survival between strains was determined by Mantel-Cox test. At the time of death, hearts were aseptically removed, fixed in formalin, and embedded in paraffin. Organs were bisected and sectioned, with every 4th section stained with hematoxylin and eosin (8 total sections per animal). Sections were imaged at 40x magnification, the number of lesions for each section was recorded, and lesion size was determined using imageJ.

Results. There was no difference in median survival between animals infected with OG1RF and OG1RFΔ*madR* (22.5 v 21 hours, p=0.31), OG1RF and OG1RF*madSA202E* (22.5 v 24 hours, p=0.29), or OG1RFΔ*madR* and OG1RF*madSA202E* (21 v 24 hours, p=0.13). There was a significant difference in the number and size of cardiac lesions between the strains. Mice infected with OG1RF*madSA202E* had a significantly higher number of cardiac microlesions as compared to those infected with OG1RFΔ*madR* (Fig 1). The size of the lesions in mice infected with OG1RF*madSA202E* was also significantly larger than those in OG1RF wild type (Fig 1).

Figure 1. Cardiac microlesions in a mouse peritonitis model of Enterococcus faecalis infection.



Mouse hearts were removed at time of animal death, placed in formalin, and embedded in paraffin. Organs were bisected, then sectioned with every 4th section stained with hematoxylin and eosin (H&E, 8 sections per animal). H&E stained sections were imaged at 40x magnification, the number and size of lesions was determined for 48 sections per strain. Representative cardiac microlesions (arrow) are shown for A) E. faecalis OG1RF, B) OG1RFΔmadR and C) OG1RFmadSA202E, scale bar 20 μm. D) The number of microlesions observed in each section, and E) the area of the lesions for each strain are shown above, differences in means were determined with one way ANOVA using Tukey's test for multiple comparisons. ns, not significant.

Conclusion. Changes in MadRS did not impact overall survival in mice, but did alter the number and size of cardiac microlesions. Further experiments are needed to determine if these changes could adversely affect therapy or rates of relapse.

Disclosures. **William R. Miller, MD** , **Entasis Therapeutics** (Scientific Research Study Investigator)**Merck** (Grant/Research Support) **William R. Miller, MD** , Entasis (Individual(s) Involved: Self): Scientific Research Study Investigator; Merck (Individual(s) Involved: Self): Grant/Research Support **Cesar A. Arias, M.D., MSc, Ph.D., FIDSA**, **Entasis Therapeutics** (Grant/Research Support)**MeMed Diagnostics** (Grant/Research Support)**Merk** (Grant/Research Support)

1003. Cytokine Levels in Sepsis and TNFα Association with Mortality but not Sepsis Severity or Infection Source: a Systematic Review and Meta-analysis

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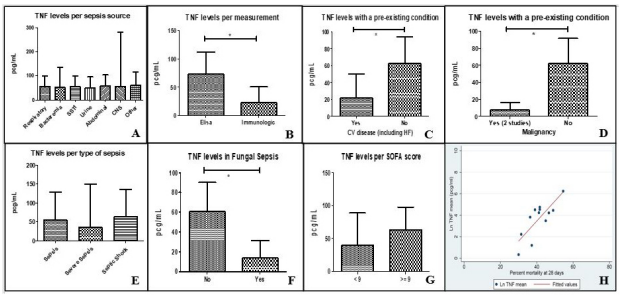
Session: P-56. Microbial Pathogenesis

Background. Sepsis is a global health problem associated with significant morbidity and mortality and is attributed to a "cytokine storm.". However, anti-cytokine therapies have failed to lower sepsis mortality in clinical trials. Linking cytokine excess to sepsis pathogenesis requires quantification of cytokine levels in sepsis. This systematic review and meta-analysis characterizes levels of key cytokines in the circulation of sepsis patients and relates TNFα levels to mortality and patient characteristics.

Methods. Medline, Embase, Cochrane Library, and Web of Science Core Collection databases were searched from 1946 to May 2020 for studies in English disclosing cytokine levels in sepsis. Keywords included sepsis, septic shock, purpura fulminans, and tumor necrosis factor (TNF)α. We related cytokine amounts to 28-day mortality. Data analyses were performed using a random-effects model to estimate pooled odds ratios (OR) and 95% confidence intervals (CI). This systematic review is registered in PROSPERO under number CRD42020179800.

Results. A total of 3656 records were identified. After exclusions, 103 studies were included. Among these studies, 72 disclosed TNFα levels, 25 showed interleukin (IL)-1β levels, and 6 presented interferon (IFN)γ levels. The pooled estimate mean TNFα concentration in sepsis patients was 58.4 pg/ml (95% CI, 39.8-85.8 pg.ml; I^2 = 99.4%). Pooled estimate means for IL-1α and IFNγ in sepsis patients were 21.8 pg/ml (95% CI, 12.6-37.8 pg.ml; I^2 =99.8%) and 63.3 pg/ml (95% CI, 19.4-206.6 pg/ml; I^2 = 99.7%), respectively. Elevated TNFα concentrations were associated with increased 28-day mortality (P=0.001). In a subgroup analysis, TNFα levels did not relate to sepsis source, sepsis severity, or sequential organ failure assessment (SOFA) score (figure 1). In a metaregression, TNFα associated with age, percentage of females and mortality at 28 days.

Figure 1: A: TNFa levels according to sepsis source. B: TNFa levels according to measurement technique. C: TNFa levels according to presence or absence of cardiovascular disease. D: TNFa levels according to presence or absence of malignancy. E: TNFa levels according to sepsis severity. F: TNFa levels in fungal compared to other causes of sepsis (Yes=fungal sepsis; No= Other types of sepsis). G: TNFa levels according to SOFA score. H: TNFa levels and mortality at 28 days.



Conclusion. We presented levels of TNFα, IL-1β, and IFNγ in human sepsis and showed that TNFα elevations are associated with sepsis mortality. TNFα concentrations did not correlate with sepsis severity. We believe the concept that elevated cytokines cause sepsis should be revisited in the context of these data.

Disclosures. **All Authors**: No reported disclosures

1004. Cladophora in Lake Michigan May Serve as Important Reservoirs for Antibiotic-Resistant Bacteria

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