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998. Forward and Reverse Translational Approaches to Predict Efficacy of the Neutralizing Respiratory Syncytial Virus (RSV) Antibody MK-1654

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Session: P-56. Microbial Pathogenesis

Background. MK-1654 is a respiratory syncytial virus (RSV) F glycoprotein neutralizing monoclonal antibody (mAb) with an extended half-life in late development to prevent RSV infection in infants. Neutralizing mAbs, like MK-1654, have great potential for prophylaxis against viral infection. However, well-validated approaches for clinical dose and efficacy predictions are lacking.

Methods. Summary-level literature data from RSV prevention studies were used in a model-based meta-analysis (MBMA) to describe the relationship between RSV incidence rates and serum neutralizing antibody (SNA) titer. The model was validated using viral challenge experiments in cotton rats and phase 3 RSV-A efficacy results in infants for an anti-RSV F mAb, REGN-2222. A phase 2b human RSV challenge study (HCS) in adults was also conducted with MK-1654. Participants (N=70) received 100, 200, 300, or 900 mg of MK-1564 or placebo and were challenged intranasally with RSV 29 days later. RSV viral load and symptomatic infection were monitored. Data from the HCS were compared to model predictions. The MBMA was used to predict efficacy of MK-1654 in a virtual population of pre- and full- term infants.

Results. The relationship between SNA titer and RSV incidence rate defined using the viral load data from the cotton rat approximated the relationship identified for infants from the clinical MBMA. The MBMA was quantitatively consistent with the phase 3 efficacy results against RSV A for REGN-2222. In the HCS, RSV nasal viral load measured by RT-qPCR and quantitative culture as well as symptomatic infections were decreased in MK-1654 recipients compared to placebo. Incidence rates of RSV infection in the HCS were also consistent with MBMA predictions. The model-based clinical trial simulations for MK-1654 indicated a high probability of substantial efficacy against RSV-associated medically attended lower respiratory tract infection (>75% for 5 months) for doses ≥75 mg.

Conclusion. Our MBMA successfully quantified the relationship between RSV SNA and clinically relevant endpoints, including lower respiratory tract infection in infants. MBMA-based efficacy predictions support continued development of the MK-1654 antibody for the prevention of RSV in infants.

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999. Nasal Mucosal Cytokines: Potential Biomarkers for Pediatric Pneumonia Severity and Etiology

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Session: P-56. Microbial Pathogenesis

Background. Community acquired pneumonia (CAP) is a leading cause of mortality in children < 5 years, but our understanding of disease pathogenesis remains limited. The objective of this study was to define the local host immune response in the respiratory tract by measuring nasal mucosal cytokine (NMC) concentrations (conc.). We hypothesized that NMC represent a potential biomarker to help assessing disease severity and pathogen classification.

Methods. We leveraged nasopharyngeal (NP) samples and clinical data from an observational multicenter study [Children's Hospital's Initiative for Research in Pneumonia (CHIRP)] conducted between 2015 and 2018. We measured conc. of 92 NMC using the Olink immunoassay. NMC conc. were compared by severity-defined by need for hospitalization, mild (outpatient) and severe (inpatient), and by identified pathogen using Mann-Whitney U test.

Results. This substudy included 182 children with CAP (mild=61; severe=121) and 30 healthy controls (HC). The pathogens identified included: 101 viruses; 32 bacteria (pyogenic=10; atypical=22); 12 with >1 pathogen; and 37 with no pathogen. Children with severe CAP had greater CCL23 and MCP-3 conc. than those with mild disease (p=0.012; p=0.011 respectively). When comparing NMC profiles of children with CAP of viral and bacterial etiology, the viral group had greater conc. of proinflammatory cytokines IL-6 and TNF, (p=0.0002; p=0.0098 respectively). Further subgroup analysis showed that CAP secondary to influenza virus had greater conc. of IL-6, TNF, and antiviral INF-γ and IP-10 compared with CAP caused by pyogenic bacteria. IL-6 and MCP1-4 were significantly increased in the influenza group compared to the atypical bacteria group.

Quantification of NMC in children with CAP based on disease severity

	Mild CAP (n=61)	Severe CAP (n=121)	
	Median concentration NPX	Median concentration NPX	P value
CCL23	1.537	1.877	0.0126
MCP-3	0.8831	1.261	0.0112

NMC. nasal mucosal cytokine; CAP: community acquired pneumonia; NPX: normalized protein expression, arbitrary unit used in Olink assay that is log 2 scale. Mann-Whitney test was used to determine differences between mild and severe pneumonia

Quantification of NMC in children with CAP based on pathogen classification

	Bacterial CAP (n=32)	Viral CAP (n=101)	
	Median Concentration NPX	Median Concentration NPX	P value
IL-6	3.284	4.768	0.0002
LAP TGF-b	1.522	2.301	0.0017
PD-L1	3.286	4.714	<0.0001
IL-17C	1.767	2.394	0.0040
uPA	7.699	8.914	0.0001
MCP-2	3.404	5.159	0.0021
IL-1 alpha	1.354	2.015	0.0059
OSM	4.912	7.125	0.0016
IL-18 R1	6.59	7.68	0.0005
TNF	0.2548	1.526	0.0098
AX1N1	3.696	2.969	0.0089
CXCL5	9.610	11.78	0.0023
CX3CL1	1.543	2.26	0.0187
LIF-R	0.8966	1.172	0.0081

NMC: nasal mucosal cytokine; CAP: community acquired pneumonia; NPX: normalized protein expression, arbitrary unit used in Olink assay that is log 2 scale. Mann-Whitney test was used to determine differences between bacterial CAP and viral CAP.

Conclusion. Children with severe CAP had higher monocyte chemoattractant NMC conc. than children with mild disease. Children with viral CAP, particularly influenza, had a more robust mucosal response including both proinflammatory and antiviral NMC than children with bacterial CAP. These findings show differences in NMC conc. based on etiology and disease severity. Further studies are needed to determine whether NMC are reliable predictive biomarkers of CAP etiology and severity.

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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.

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

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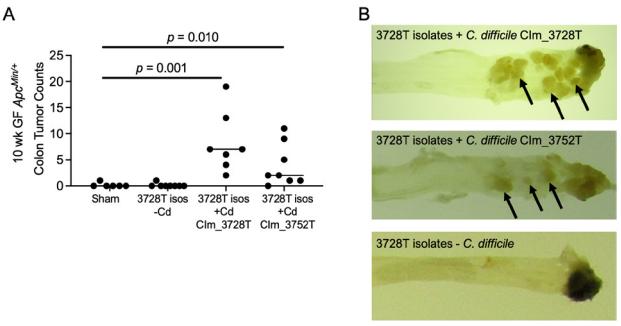
Session: P-56. Microbial Pathogenesis

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.

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