Pathogenicity of Plasmid Positive and Negative Chlamydia trachomatis in a Macaque Model of Ocular and Genital Tract Diseases

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BACKGROUND

Previous studies demonstrated that a *Chlamydia* trachomatis plasmid negative OCULAR strain (A/P-) resulted in attenuation of infection and pathology, paired with immune stimulation that produced protective immunity in a monkey ocular model.

> C. trachomatis Plasmid-Cured Trachoma Strain A2497P-Highly Attenuated for Monkey Eye ari L et al. JEM 2011;208:2217–2223.

OBJECTIVE

To assess a **plasmid-negative GENITAL strain (D/P-)** in the pigtailed macaque model of chlamydial reproductive tract infection, comparing infection and pathology to a **plasmid-positive GENITAL strain (D/P+)**.

METHODS

Groups of six macaques were cervically challenged with C. trachomatis D/P+ or D/P-. All animals were followed for infection, circulating antibody, local immunity, and tissue inflammation. Upon spontaneous clearance of cervical infection, each animal underwent repeated challenges with matched strains to drive upper reproductive tract disese. The same strains were similarly compared in the macaque **OCULAR** infection model.



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RESULTS

Similar rates and duration of chlamydial genital infection were documented in both the P+ and P- challenged macaques. Likewise, serum and local antibodies were similar.

	D/P	+		Inf	ieo	ctio	on	Sta	atı	IS,	LO	cal	a	nd	Cir	'CU	llat	tin	g A	\n	tib	od	ies	
			D/P+															D/P+	D/P+	D/P+	D/P+	D/P+		
		*							<u>.</u>								2						2	
	Week	Wk -2	0	1	2	3	4	6	8	11	13	15	17	19	21	23	25	27	28	29	30	31	40	
	culture		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	9180	1	13	64	
	NAAT		٠	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	POS	POS	POS	POS	POS	
15	local IgG		٠	•	•	•	•	•	nd	•	•	•	•	•	•	•	nd	•	•	•	•	•	•	
100	slgA		٠	•	•	•	•	•	nd	•	•	•	•	•	•	•	nd	•	•	•	•	•	•	
Ā	Ct lgG	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	1:32	1:64	
		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	1:64	1:32	
		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	2280	240	
			•	POS		•	•	•	•	•	•	•	•	•	•	•	•	•	POS	POS	POS	2300 POS	240 POS	
Σ				•		•	•	•	nd	•	•	•	•	•	•	•	nd	•	•	•	•	•	•	
019	slaA		•	•	•	•	•	•	nd	•	•	•	•	•	•	•	nd	•	•	•	•	•	•	
A1(Ct IgG	•	•	•	•	1:8	•	•	•	•	•	•	•	•	•	•	•	•	•	1:32	1:32	1:16	1:64	
	Ct IgM	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	1:16	l
	Ct IgA	•	٠	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	1:16	
	culture		•	•	71	13	19	850	544	3060	3	38	•	•	•	•	•	•	•	126	6	•	•	l
	NAAT		٠	POS	POS	POS	POS	POS	POS	POS	POS	POS	•	•	•	•	•	•	•	POS	POS	POS	•	l
539	local IgG		٠	•	•	•	•	1:128	nd	1:64	1:128	1:128	•	1:256	•	•	nd	•	•	•	1:512	•	1:16	l
122	slgA		•	•	•	•	•	•	nd	•	•	•	•	•	•	•	nd	•	•	•	•	•	•	l
Ā	Ct lgG	•	•	•	1:64	1:128	1:256	1:128	1:128	1:128	1:256	1:128	1:128	1:256	1:256	1:128	1:256	1:256	1:256	1:512	1:512	1:512	1:128	l
		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	l
		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	0.25	•	•	•	•	l
			•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	935 POS	40 POS	POS	•	•	l
0								•	nd								nd	•	FUS	FUS	FUS	•		l
224	slaA		•	•		•	•	•	nd	•	•	•	•	•	•	•	nd	•	•	•	•	•	•	l
A12	Ct IgG	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	1:16	1:64	1:128	1:32	l
	Ct IgM	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	l
	Ct IgA	•	٠	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	l
	culture		٠	33	23	70	1	24	•	•	•	•	•	•	•	•	•	•	16	8	51	32	•	l
	NAAT		٠	POS	POS	POS	POS	POS	POS	•	•	•	•	•	•	•	•	•	POS	POS	POS	POS	•	l
69	local IgG		•	•	•	•	•	•	nd	1:16	•	•	•	•	•	•	nd	•	•	•	1:32	1:128	•	l
052	slgA		•	•	•	•	•	•	nd	•	•	•	•	•	•	•	nd	•	•	•	•	•	•	l
	Ct lgG	•	٠	•	•	1:64	1:128	1:128	1:64	1:32	1:32	1:32	1:16	1:16	1:16	1:16	1:8	1:16	1:32	1:64	1:128	1:128	1:32	l
		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	l
		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	l
			•	3/5		4/	40	29	35		4	9	• DOS		•	•	•	•	19		80	● DOS	•	l
2			-	-03	-03	105	100	FU3	nd	FU3	F03	FU3	FU3	F03			nd		FU3	FU3	P03	F03	-	
08	sla		-						nd								nd							
205	Ct laG	•	•	•	•	1:64	1:128	1:128	1:128	1:128	1:64	1:64	1:64	1:128	1:128	1:64	1:32	1:64	1:64	1:256	1:256	1:256	1:64	
	Ct IgM	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
	Ct IgA	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	

Tissue inflammation, graded by gross observation during laparoscopic procedures and by tissue histology, yielded no discernible patterns to disease pathology between P+ and P- strains.

Visual Assessment of Inflammation Summary (each laparoscopy and necropsy)

P+ P-	Norm a (No adhesion	Normal URT adhesions/no friability) Mild URT Involvement (Increased erythema, edema, stiff tubes, thick mesosalpinx)		Interm (Stiff tube(s) boo pinx, early adhe	PID (Peritubal/periadne widespread or fibro adhesions, friable tis		
Baseline	A10015 A10191 A12239 A12249 L05269 Z08082	A10217 A12237 A12244 M06119 Z06366 Z08122					
Weeks 7/8	A10191	A10217 Z06366	A10015 A12239 A12249 L05269	A12237 Z08122	Z08082	A12244 M06119	
Wks24/25	A10191	A10217 Z06366 Z08122	A10015 A12249 L05269	M06119	A12239 Z08082	A12237 A12244	
Week 39		A10217	A10015 A10191 A12239 A12249 L05269	A12237 A12244 M06119 Z06366 Z08122	Z08082		
eks 41/42	L05269	Z06366	A10015 A12239 A12249	A10217 A12244 M06119	A10191 Z08082		





Because A/P+ and A/P- ocular strains in macaques exhibited dramatic differences in infectivity and pathology in the eye, we ocularly challenged animals with D/P+ and D/P-.

Chlamydial Burden



Inflammatory Response: Histology and Immunohistochemistry (IHC) Stains (avg cell counts*)

	LE			
Arms:	D/P+	D/P-	Naïve	D/
PMNs	2.5	0.0	0.8	0
Lymphocytes	22.0	13.0	10.6	14
Plasma Cells	4.0	3.3	1.5	4,
CD68 (KP1)	3.3	6.2	5.4	3
CD4	9.7	7.5	0.0	7
CD8	19.9	11.5	1.8	11
CD20	3.6	2.8	2.0	2
			-	

H&E

IHC

H&E

IHC

MBR	RIA	RIGHT FIMBRIA			
D _	Naïve	D/P+	D/P-	Naïve	
0	0.8	0.6	0.0	0.0	
.0	10.6	14.4	11.2	5.0	
3	1.5	4.3	3.7	0.9	
2	5.4	3.3	5.2	na	
5	0.0	7.0	6.2	na	
.5	1.8	11.6	9.9	na	
8	2.0	2.6	3.1	na	

	UTERUS					
D/P+	D/P-	Naïve				
0.2	1.6	0.3				
8.9	7.3	4.8				
0.8	0.5	0.0				
5.6	7.4	na				
4.5	4.9	na				
5.5	4.5	na				
1.2	1.4	na				

ENDOCERVIX					
D/P+	D/P-	Naïve			
14.8	7.4	6.2			
1.6	1.3	6.2			
0.4	0.4	0.8			
5.3	2.5	2.2			
1.1	1.5	0.0			
2.8	2.0	0.0			
0.3	0.6	0.0			

ïve
.0
.9
.2
na
na
na
na

E	CTOCERV			
)/P+	D/P-	Naïve	D/P+	
0.0	0.4	3.5	0.0	
21.3	17.8	20.0	26.5	
5.1	5.0	1.4	5.2	
1.8	3.7	2.8	2.9	
13.5	16.8	0.0	18.7	
25.4	18.0	0.0	25.7	
4.0	9.0	2.0	5.6	

VAGINA						
)/P+	D/P-	Naïve				
0.0	0.5	0.8				
26.5	23.6	9.0				
5.2	6.7	1.6				
2.9	5.0	1.0				
18.7	20.0	1.0				
25.7	21.0	2.8				
5.6	6.8	1.0				

*Displayed values are calculated as the average value of the mean number of positively stained cells (5 non-adjacent HPFs) for all macaques in the study arm

Naïve = Controls (n=1-3 animals) **D/P-** (n=6) **D/P+** (n=6) Arms:

SUMMARY

D/P

0.9

1.4

1.9

3.4

0.8

Arms

PMNs

CD4

CD8

CD20

Lymphocytes

Plasma Cells

CD68 (KP1)

Unexpectedly, no differences in infectivity or pathology were observed between the D/P+ and D/Pstrains; each produced similar infection kinetics with ocular disease, characterized by conjunctival hyperemia and follicle formation.

Ocular Clinical Disease



CONCLUSIONS

- Unlike the ocular strain, the plasmid negative genital strain is not attenuated in either genital or ocular macaque infection models.
- 2 This suggests that genetic determinants unrelated to the plasmid play a dominant role in the pathogenesis of urogenital strains.

