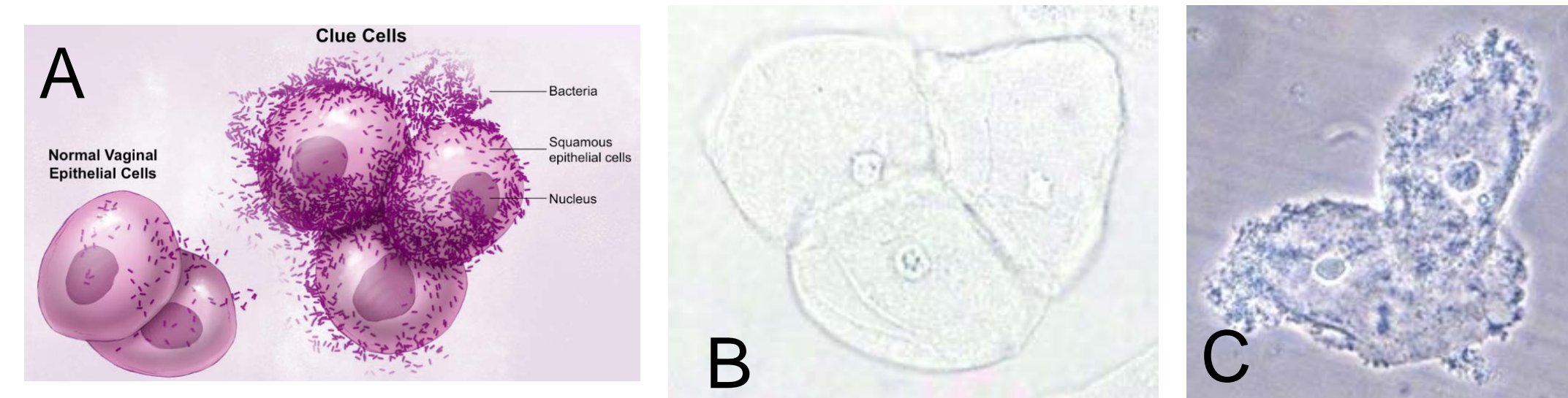# Chemokine Profiling of Vaginal Epithelial Cells Exposed to *Gardnerella vaginalis*

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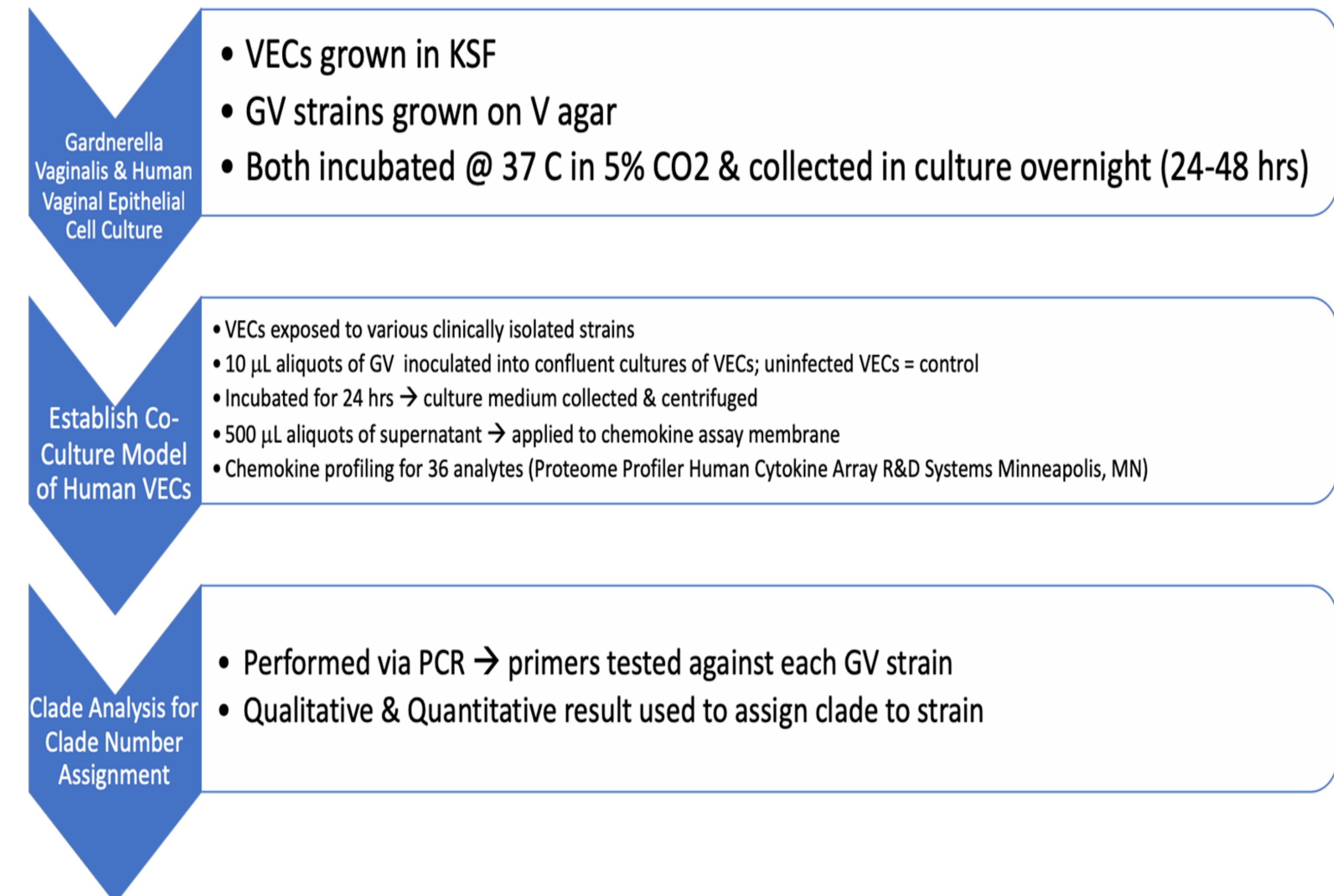Marian University College of Osteopathic Medicine[1]

Introduction

Gardnerella vaginalis (GV) is associated with bacterial vaginosis (BV) and when present in abundance, causes a dysbiosis characterized by increased pH, presence of clue cells, malodorous discharge, and a diversity of bacterial species other than *Lactobacillus*. BV predisposes to preterm labor and increased susceptibility to sexually transmitted infections. The potential role of cytokines elaborated by the vaginal epithelium may impact BV symptoms and hence, is the topic of this research.

A. Cartoon depiction of normal vaginal epithelial cells versus VECs covered in GV, called clue cells. B. Normal vaginal epithelial cells under light microscope. C. Clue cells under light microscope.

Cytokine/Chemokine Upregulation

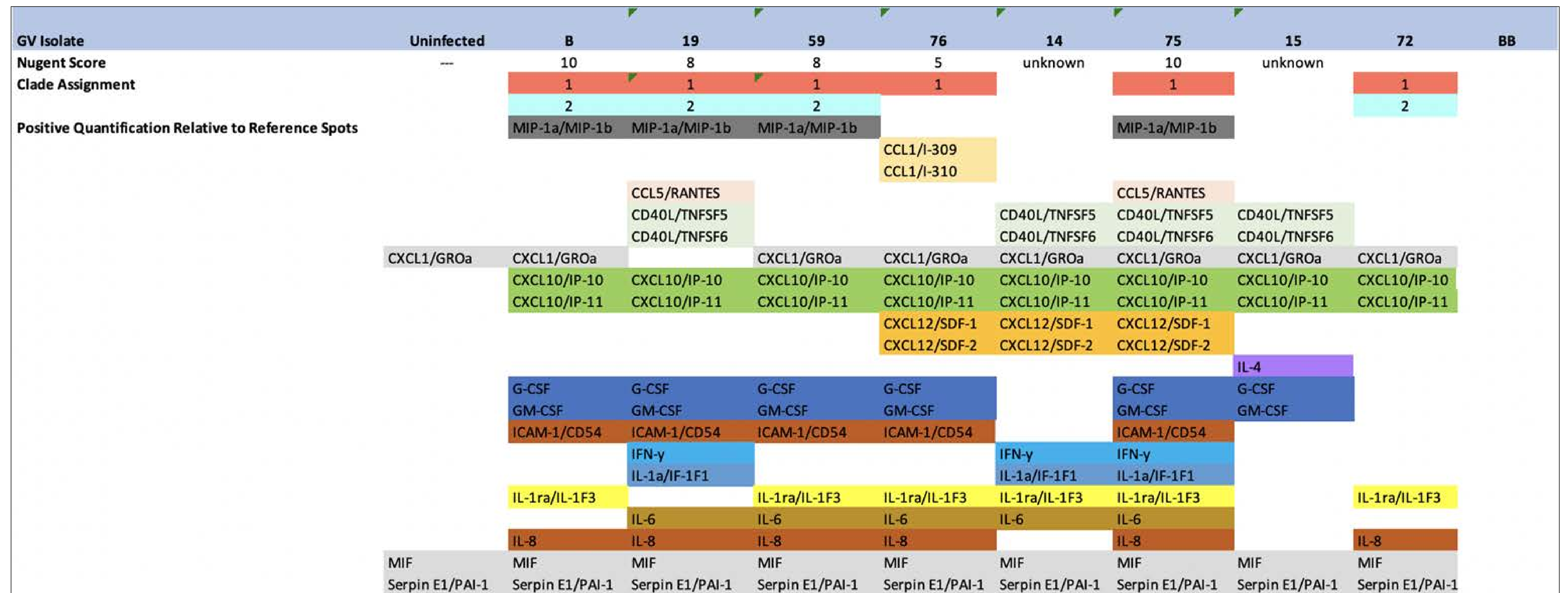| GV Isolate	Uninfected	B	19	59	76	14	75	15	72	BB
Nugent Score	---	10	8	8	5	unknown	10	unknown		
Clade Assignment		1	1	1	1		1		1	
		2	2	2					2	
Positive Quantification Relative to Reference Spots		MIP-1a/MIP-1b	MIP-1a/MIP-1b	MIP-1a/MIP-1b			MIP-1a/MIP-1b			
					CCL1/I-309					
					CCL1/I-310					
			CCL5/RANTES				CCL5/RANTES			
			CD40L/TNFSF5			CD40L/TNFSF5	CD40L/TNFSF5	CD40L/TNFSF5		
			CD40L/TNFSF6			CD40L/TNFSF6	CD40L/TNFSF6	CD40L/TNFSF6		
	CXCL1/GROa	CXCL1/GROa		CXCL1/GROa	CXCL1/GROa	CXCL1/GROa	CXCL1/GROa	CXCL1/GROa	CXCL1/GROa	
		CXCL10/IP-10	CXCL10/IP-10	CXCL10/IP-10	CXCL10/IP-10	CXCL10/IP-10	CXCL10/IP-10	CXCL10/IP-10	CXCL10/IP-10	
		CXCL10/IP-11	CXCL10/IP-11	CXCL10/IP-11	CXCL10/IP-11	CXCL10/IP-11	CXCL10/IP-11	CXCL10/IP-11	CXCL10/IP-11	
				CXCL12/SDF-1	CXCL12/SDF-1	CXCL12/SDF-1	CXCL12/SDF-1			
				CXCL12/SDF-2	CXCL12/SDF-2	CXCL12/SDF-2	CXCL12/SDF-2			
							IL-4			
		G-CSF	G-CSF	G-CSF	G-CSF		G-CSF	G-CSF		
		GM-CSF	GM-CSF	GM-CSF	GM-CSF		GM-CSF	GM-CSF		
		ICAM-1/CD54	ICAM-1/CD54	ICAM-1/CD54	ICAM-1/CD54		ICAM-1/CD54			
		IFN-y				IFN-y	IFN-y			
		IL-1a/IF-1F1				IL-1a/IF-1F1	IL-1a/IF-1F1			
	IL-1ra/IL-1F3		IL-1ra/IL-1F3	IL-1ra/IL-1F3	IL-1ra/IL-1F3	IL-1ra/IL-1F3			IL-1ra/IL-1F3	
		IL-6		IL-6	IL-6	IL-6				
		IL-8	IL-8	IL-8	IL-8		IL-8		IL-8	
	MIF	MIF	MIF	MIF	MIF	MIF	MIF	MIF	MIF	
	Serpin E1/PAI-1	Serpin E1/PAI-1	Serpin E1/PAI-1	Serpin E1/PAI-1	Serpin E1/PAI-1	Serpin E1/PAI-1	Serpin E1/PAI-1	Serpin E1/PAI-1	Serpin E1/PAI-1	

Methods

We hypothesized that host-derived chemokine expression will correlate with Nugent (Gram stain species diversity) severity scores obtained from women with GV colonization.

Gardnerella Vaginalis & Human Vaginal Epithelial Cell Culture
- VECs grown in KSF
- GV strains grown on V agar
- Both incubated @ 37 C in 5% CO2 & collected in culture overnight (24-48 hrs)

Establish Co-Culture Model of Human VECs
- VECs exposed to various clinically isolated strains
- 10 μL aliquots of GV inoculated into confluent cultures of VECs; uninfected VECs = control
- Incubated for 24 hrs → culture medium collected & centrifuged
- 500 μL aliquots of supernatant → applied to chemokine assay membrane
- Chemokine profiling for 36 analytes (Proteome Profiler Human Cytokine Array R&D Systems Minneapolis, MN)

Clade Analysis for Clade Number Assignment
- Performed via PCR → primers tested against each GV strain
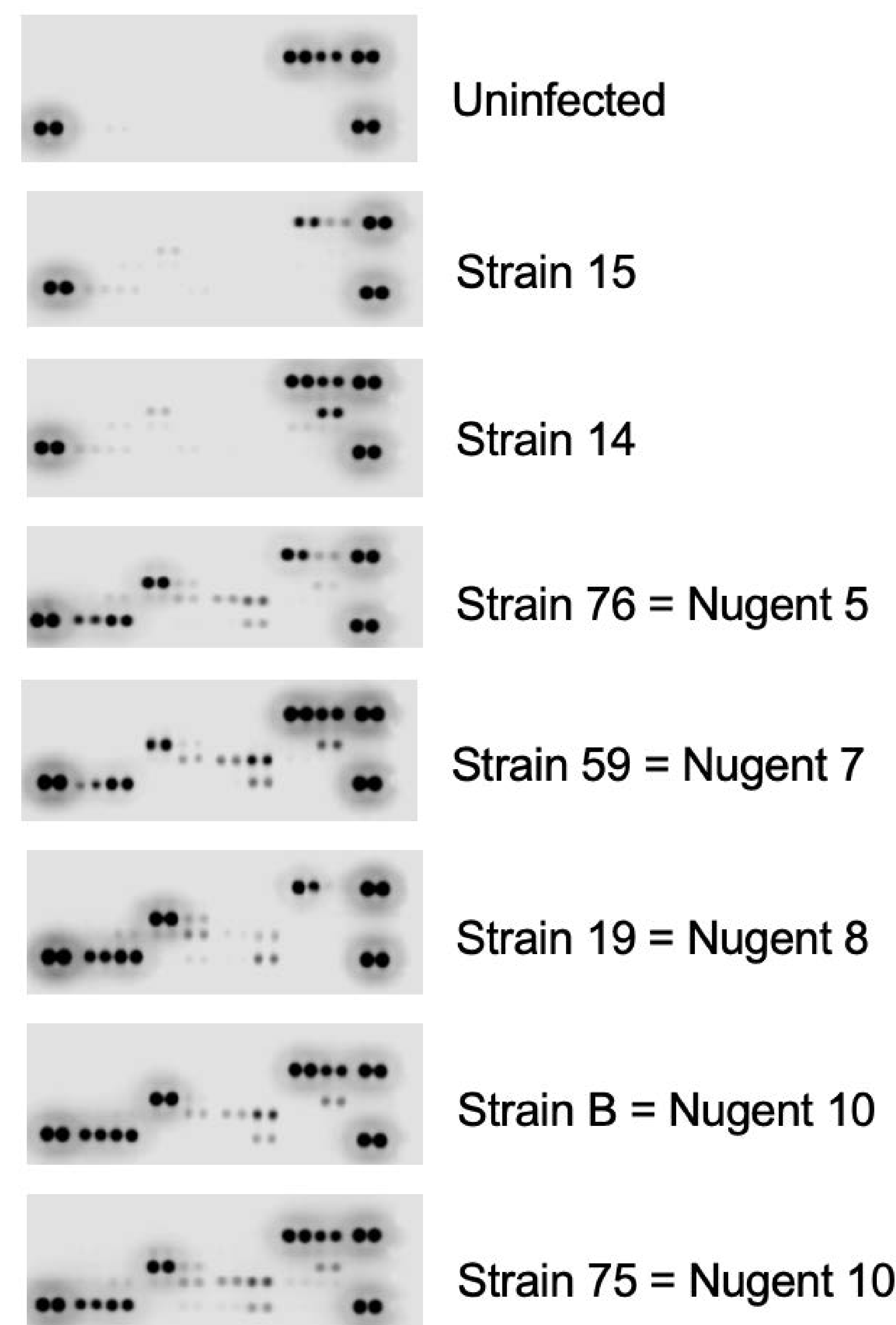- Qualitative & Quantitative result used to assign clade to strain

Results

Membrane profiling results showed that 23 cytokines and chemokines were variously upregulated following exposure of VECs to GV, while others are down-regulated relative to the control. Continued investigation will attempt to correlate Nugent scores and placement of strains into clades 1-4.

Representative Images of Cytokine Arrays

Uninfected

Strain 15

Strain 14

Strain 76 = Nugent 5

Strain 59 = Nugent 7

Strain 19 = Nugent 8

Strain B = Nugent 10

Strain 75 = Nugent 10

Conclusions

Our results indicate that co-culture with GV induces release of numerous chemokines from VECs. Future work will determine if these chemokines signal in an autocrine/paracrine manner and/or participate in the recruitment and activation of immune cells in response to GV colonization.

Acknowledgements

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