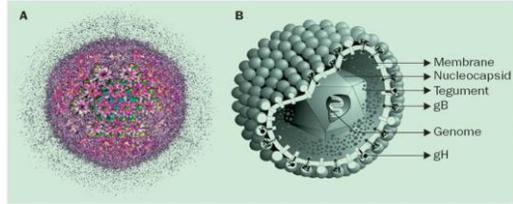


# The role of CMV in the long term outcome for older Australians, renal transplant recipients and HIV patients



## Some well known facts

- CMV is a herpes virus so it has a DNA genome
- It replicates in fibroblasts, monocytes and endothelial cells
- It can become latent and persist for a lifetime
- A subset of its genes are expressed in latently infected cells
- 80% of people in this room are CMV seropositive

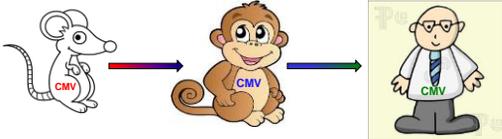


## Some trickier facts

CMV has over 200 open reading frames but most are not needed for growth in cultured cells

Many of these non-essential genes are homologues (copies) of cellular genes ...including chemokines and NK receptors. These are implicated in the ability of CMV to avoid being cleared by the immune system

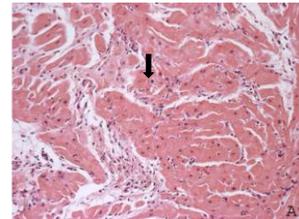
Other mammals have their own versions of CMV...which have been separate from human CMV throughout mammalian evolution.



Since each CMV has picked up similar genes from their host, they must confer a survival advantage....

## Another tricky fact....

CMV causes a lot of inflammation for a small amount of virus

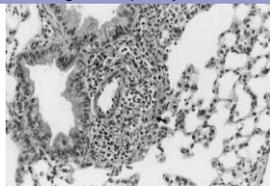


This is from a patient with CMV myocarditis

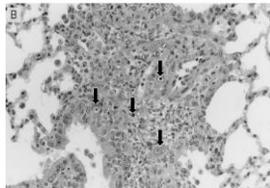


## CMV pathology varies with the immunological capacity of the host

This example is the lung of a mouse receiving cyclosporin with CMV pneumonitis...



...and a nude mouse with no T-cells has more persistent inflammation, necrosis and more infected cells.



Price & Over. Clin Immunol Immunopath 1996 80: 215-224.

## Epidemiological evidence associating CMV with diseases of aging is gathering

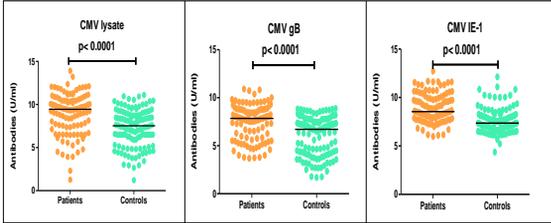
Eg: Seropositivity and higher IgG antibody levels against CMV associated with all-cause mortality and ischemic heart disease in the population based EPIC-Norfolk cohort. Clin. Inf. Dis. 2013, 56:1421-7

Q1. Is CMV associated with all diseases of aging?



...for example do older people with pulmonary disease associated with non-tuberculous mycobacteria have high levels of CMV antibodies?

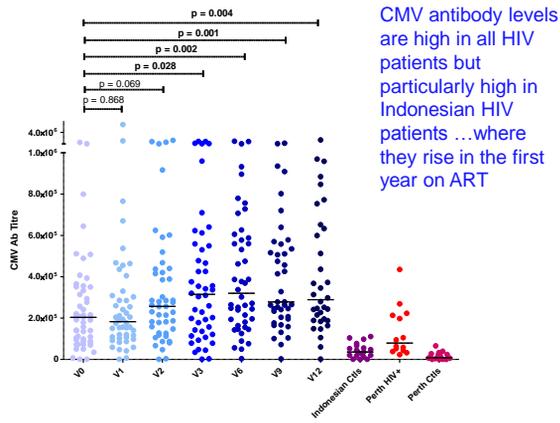
IgG reactive with whole viral lysate, glycoprotein B (gB) or Immediate Early-1 (IE-1) were measured by ELISA in plasmas from 112 NTM patients & 117 controls.



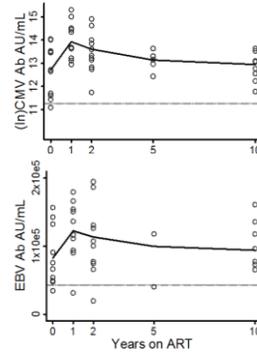
p-values determined by Mann-Whitney Test

**YES!**

Is it valid to use antibody as a measure of the lifetime burden of CMV?  
...particularly in HIV patients



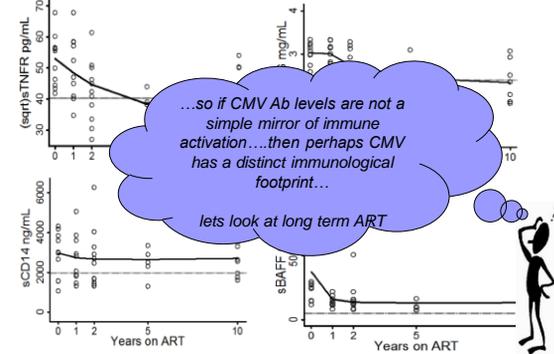
CMV and EBV antibody titres rise on ART ...and then decline slowly but stay above healthy controls



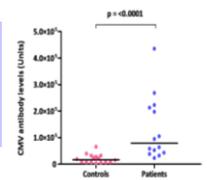
Does this reflect changes in immune activation on ART?

The rise and fall of CMV antibody did not follow plasma markers of monocyte or B-cell activation

Monocyte activation: sTNFR & sCD14      B-cell activation: Total Ig & sBAFF

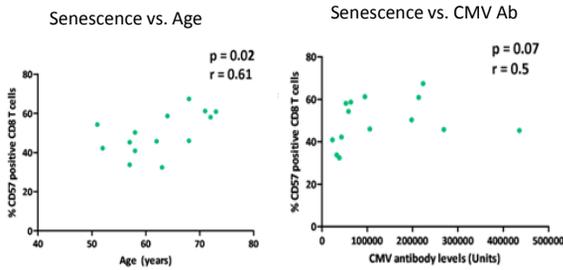


HIV patients with nadirs <200 CD4 T-cells/ $\mu$ l, a median of 14 years on ART and no identifiable CMV disease ...have high titres of CMV reactive antibody



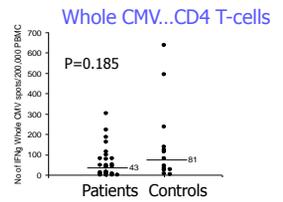
	CMV+ HIV patients	CMV- Controls	CMV- Controls
n	20	16	9
Age (years)	62 (57-68)	60 (57-62)	55 (53-59)
CMV lysate IgG (AU/ml)	94 (56-240)	20 (10-35)	0.9 (0.6-1.0)
CMV gB IgG (AU/ml)	127 (90-172)	45 (27-55)	1.9 (1.1-2.8)
CMV IE-1 IgG (AU/ml)	49 (21-166)	8 (4.3-180)	2.6 (2.0-3.7)
CMV lysate IFN $\gamma$ from CD4 <sup>+</sup> T-cells	227 (16-700)	157 (13-617)	0 (0-0.5)

### CD8 T-cell senescence correlates with CMV antibody and age in HIV patients

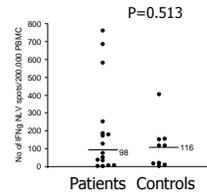


So...if antibody is a measure of CMV, then perhaps it is not protective???

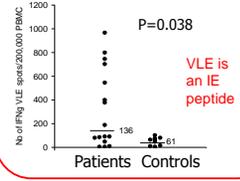
When we measured  $IFN\gamma$  responses by T-cells after 8 years on ART.....



NLV peptide...CD8 T-cells



VLE...CD8 T-cells

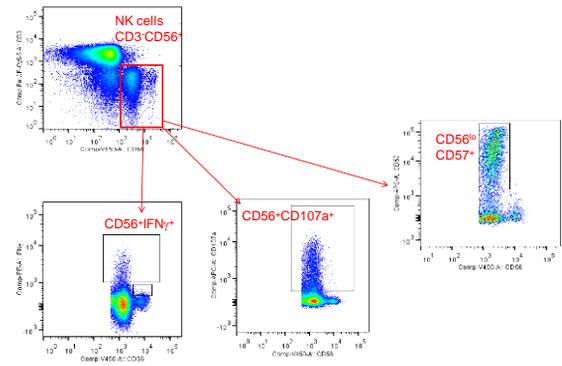


### CMV seropositivity and controlled HIV disease (~14 yrs on ART) increase T-cell differentiation towards an exhausted phenotype

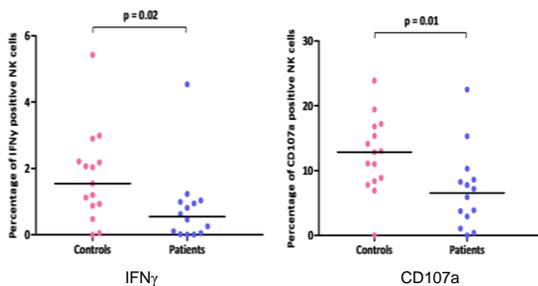
	CMV+ HIV patients	CMV + Controls	CMV- Controls
<i>n</i>	20	16	9
Age (years)	62 (50-73)	60 (50-74)	55(52-69)
CMV lysate antibody AU/L	94 (23-995)	20 (6-83)	0.8 (0.5-1.1)
CD4 <sup>+</sup> T cells [%lym]*	43 (24-77)	69 (52-84)	69 (52-80)
CD57 <sup>+</sup> [%CD4]	11 (2-75)	8 (2-26)	4.5 (1.7-7.4)
CD57 <sup>+</sup> CD45RA <sup>+</sup> CD27 <sup>-</sup> [%CD4]	1.9 (0-57)	0.4 (0.1-14)	0.02 (0-0.16)
CD8 <sup>+</sup> T cells [%lym]	48 (16-71)	22 (7-43)	21 (15-44)
CD57 <sup>+</sup> [%CD8]	47 (17-67)	40 (6.4-69)	28 (10-68)
CD57 <sup>+</sup> CD45RA <sup>+</sup> CD27 <sup>-</sup> [%CD8]	19 (4.2-53)	26 (4-49)	8 (2-19)

\*T cell subset [as % of]

### NK cells are also important in the control of CMV



### HIV infection decreases NK cell function...even after ~14 years on ART



This may impair clearance of reactivated CMV

### Controlled HIV disease (median 14 years on ART) decreases NK numbers & function and increases NK expression of CD57...

LIR-1 expression is increased by CMV...not by HIV

	CMV+ HIV patients	CMV+ controls	CMV- controls
NK cells [%]	12 [8.3-18]	17 [11-20]	17 [12-22]
$IFN\gamma$ [%NK cells] × CD56	0.80 [0.30-1.10]	1.7 [1.0-2.6]	1.0 [0.6-2.7]
LIR-1 [MF] [%NK cells]	554 [419-706]	524 [351-774]	301 [270-734]
CD57 <sup>+</sup> [%NK cells]	77 [59-83]	64 [54-66]	63 [59-65]

LIR-1 is an inhibitory receptor for NK cells

•Binds to HLA-G on a healthy cells to say "DON'T KILL ME!"

•Binds to CMV protein UL18 with 1000 times higher affinity than HLA-G

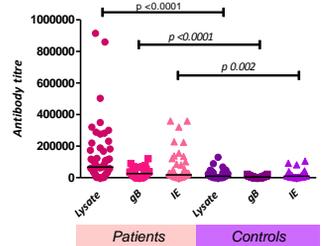
•LIR1 expression on NK cells is increased in transplant patients after CMV disease

Can we look at another group of patients with a high lifetime burden of CMV?  
 ...renal transplant recipients

We recruited 55 patients and 40 age-matched controls. All patients had been stable on therapy for >2 years

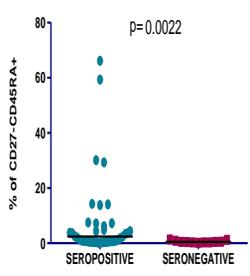


Renal transplant recipients also have high levels of antibody reactive with CMV

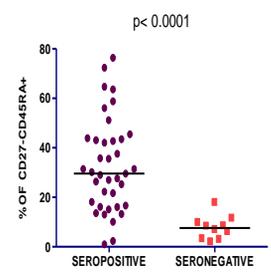


**Lysate** Mixture of CMV antigens  
**gB** Fusion protein required for infection  
**IE** Immediate Early protein secreted first during replication

CD4 T-cells

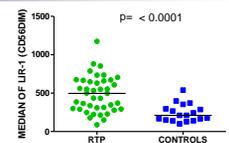


CD8 T-cells



CMV seropositive RTR have more senescent T-cells

Levels of LIR-1 on NK cells are high in RTR and proportional to levels of CMV gB antibody and DNA



Correlating NK markers with CMV gB antibody in RTR

Marker	R value	P value
NKG2C	0.30	0.03
LIR-1 (gB)	0.41	0.003
Perforin (gB)	-0.33	0.02

Correlating NK markers with CMV DNA in RTR

LIR-1 (gB)	0.37	0.007
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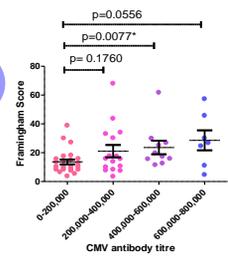
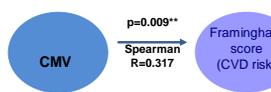
So CMV blocks the cells that are supposed to control it in RTR!

So does CMV influence cardiovascular disease?

...is this clearer in RTR and/or HIV patients?



CMV antibodies correlate with increased risk of cardiovascular disease in HIV patients....based on a study by Dr Lucette Cysique in Sydney



No component of Framingham score (gender, lipids, use of hypertensives, smoking, diabetes) associated with CMV antibody - with the exception of age

Whilst we cant correlate this with real measurements of CVD in HIV patients, we do have data from renal transplantation

Both CMV seropositivity and renal transplantation decrease arterial elasticity

	CMV+ RTR	CMV- RTR	CMV+ Controls	CMV- Controls
n	69	13	44	28
Male:Female	39:30	3:6	39:30	13:15
Age (years)	57(31-76)	53(33-62)	55(21-86)	43(31-69)
BMI	26(17-58)	30(25-40)	26(18-40)	27(20-38)
CMV lysate antibody AU/L	<b>795(56-7611)</b>	0.5(0-3.2)	299(6-1496)	0.09(0-6.6)
Arterial elasticity <b>%FMD ((B-A)/A)x100</b>	<b>3.5(0-16)</b>	5.9(2.3-13)	<b>7.6(1.4-14)</b>	9.5(4-18)
Thickness of arterial wall Left cIMT	0.66(0.4-1.3)	0.65(0.5-1)	0.65(0.4-0.9)	0.6(0.4-0.9)



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