Investigating the effects of Methylseleninic Acid on anti-HIV immunity

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

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Background

The HIV reservoir

Antiretroviral therapy (ART) has greatly enhanced life expectancy for people living with HIV; however, this treatment is not curative due to the persistence of a pool of latently infected cells known as the reservoir

Negative Expression Factor (NEF)

Nef is a non-enzymatic accessory protein encoded by HIV that downregulates MHC-1 on cells infected with HIV, enabling them to avoid CD8+ T cell mediated killing[1]

Methylseleninic Acid (MSA)

MSA is an organic selenium-based compound with immunomodulatory properties such as the ability to upregulate MHC-1 expression in cancer cells[2]

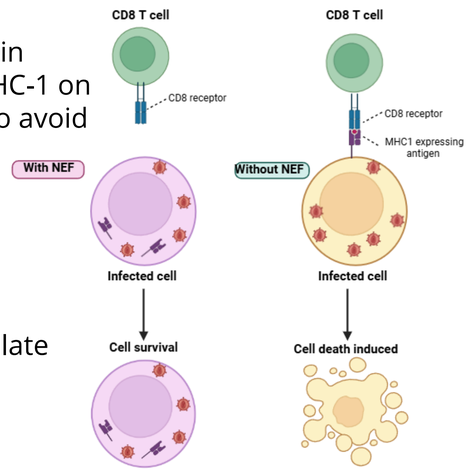

Fig1.NEF mediated downregulation of MHC-1 and subsequent impacts on CD8+ T cell mediated killing

Hypothesis and Aims

Hypothesis

Treatment with MSA will enhance anti-HIV immunity through improved immune detection of infected cells

Aims

1.Determine whether MSA increases MHC-1 in the presence of Nef

2.Determine whether MSA causes off target effects in immune effector cells

Methods

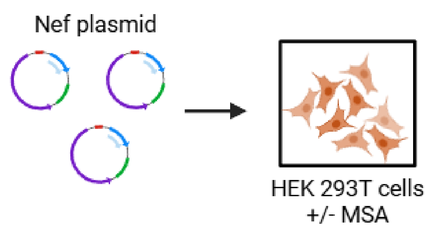

Fig2.Nef transfection model: HEK-293T cells were transfected with Nef plasmid and treated with MSA for 24 hours. Results were analysed via conventional flow cytometry

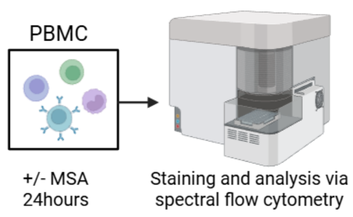

Fig3. Phenotyping assay: Primary cells from uninfected donors were pre-treated with MSA for 24 hours. Changes to phenotype were then analysed via spectral flow cytometry

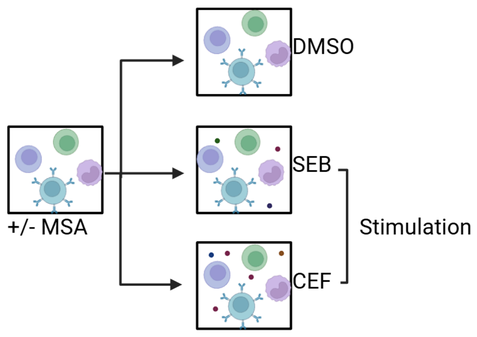

Fig4. T cell stimulus assay: Primary cells from uninfected donors were pre-treated with MSA for 24 hours. Cells were then either stimulated with staphylococcus enterotoxin B (SEB) or a peptide pool containing antigens from Cytomegalovirus, Eppstein-Barr Virus and Influenza (CEF). Results were analysed via spectral flow cytometry

References

1. Collins et al., Nature (1998)
2. Lennicke et al., Oncoimmunology (2017)

Results 1.MSA treatment can moderately combat downregulation of MHC-1 by Nef

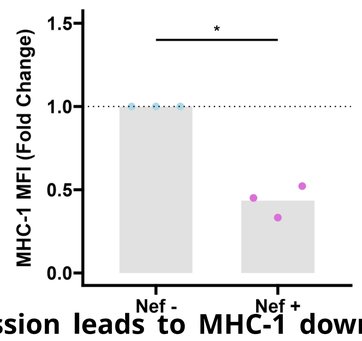

Fig5.Nef expression leads to MHC-1 downregulation in HEK-293T cells Downregulation of MHC-1 expression following transfection with Nef relative to untransfected cells. Data was acquired via flow cytometry and comprises of three independent experiments. Independent experiments are represented by dot plots and grey columns represent the mean value. Statistical comparisons for MHC-1 MFI were made with a paired T test.*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

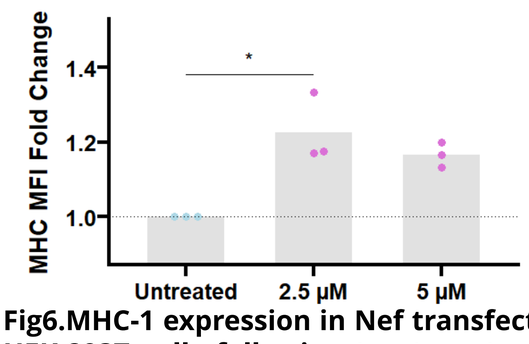

Fig6.MHC-1 expression in Nef transfected HEK-293T cells following treatment with MSA for 48 hours Data was acquired via flow cytometry and comprises of three independent experiments. Independent experiments are represented by dot plots and grey columns represent the mean value

2.MSA minimally impacts NK cell phenotype

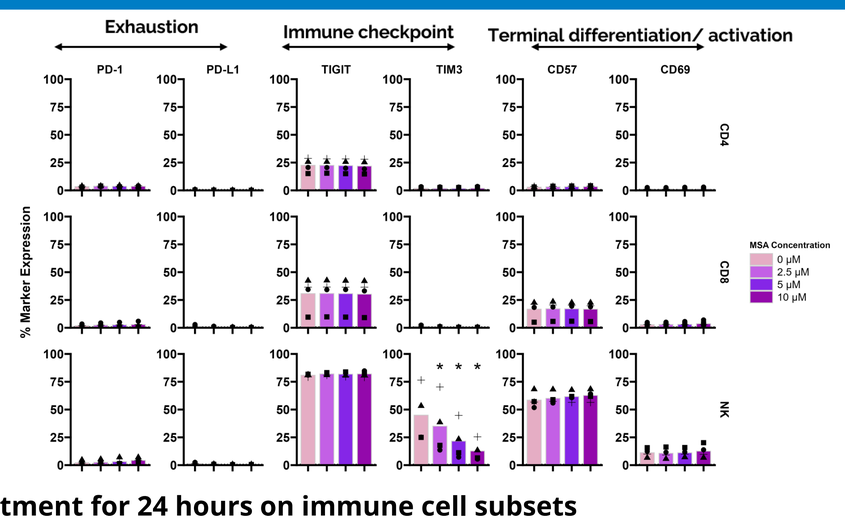

Fig7.Effect of MSA treatment for 24 hours on immune cell subsets

Changes to expression of CD57, CD69, PD1, PDL1, TIGIT and TIM3 by cell type following treatment with MSA at 2.5µM, 5µM and 10µM over 24hours. Data are represented as % positive cells for a given marker. Data was acquired via flow cytometry and comprises of four independent donors. Independent donors are represented by dot plots with columns representing the mean value. Statistical comparisons were made via repeated measures Anova with post hoc paired T test and Benjamini-Hochberg corrections. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Results 3.MSA minimally impacts T cell response to antigen stimulation

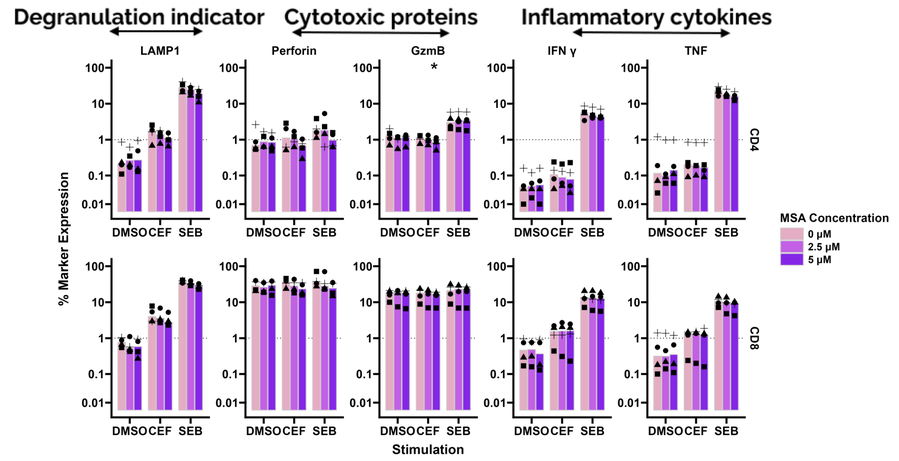

Fig8.Effect of MSA treatment on response to stimulus by cell type

Changes to expression of Granzyme B, IFN, LAMP-1, Perforin and TNF by cell type and stimulation condition following treatment with indicated concentrations of MSA for 24 hours. Data are represented as % positive cells for a given marker. Data was acquired via flow cytometry and comprises experiments four independent donors. Independent donors are represented by dot plots with columns representing the mean value. Statistical comparisons were made via repeated measures Anova with post hoc paired T test and Benjamini-Hochberg corrections. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Conclusions

MSA was able to moderately upregulate MHC-1 in cell lines despite the presence of Nef. This effect may be biologically significant

MSA appeared to cause minimal defects to immune effector cell phenotype and response to antigen stimulation

MSA may show potential clinical significance as a HIV therapeutic, but further investigations are warranted