Coxsackievirus A21 (CVA21): Immune cell interactions and oncolytic activity in leukaemia

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Introduction

Coxsackie virus CVA21 is a small, non-enveloped virus with a positive-sense RNA genome which can cause mild cold-like symptoms. It has been investigated as an oncolytic agent for the treatment of melanoma, prostate cancer, breast cancer and multiple myeloma.

The principle receptor for CVA21 is intercellular adhesion molecule-1 (ICAM-1/CD54), though decay-accelerating factor (DAF/CD55) is also reported to be a binding factor for this virus. Efficient infection of cancer cells is thought to be mediated by the overexpression of ICAM-1 or DAF on the cell surface.

The first clinical study of systemic delivery of CVA21 is in development. However, as yet there have been no studies of the effect of CVA21 on the immune system.

Aims

- To investigate the expression of ICAM-1 and DAF on peripheral blood mononuclear cells (PBMC)
- To test the effects of CVA21 on PBMC viability
- To investigate phenotypic activation of PBMC by CVA21
- To test interferon secretion by PBMC in response to CVA21
- To test the oncolytic potential of CVA21 against human acute myeloid leukaemia (AML) and chronic lymphocytic leukaemia (CLL)

PBMC variably express the receptors for CVA21 but are not killed by the virus

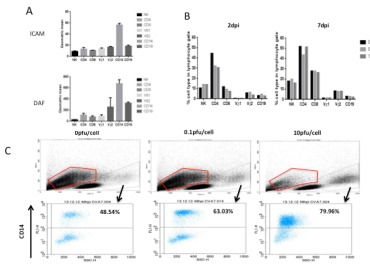

Figure 1: A) Expression of ICAM-1 and DAF by components of PBMC; B) % of NK, B and T cells within PBMC 2 and 7 days post infection (DPI); C) Increase in % of CD14+ monocytes at 2 DPI (black gate), together with fewer dead/dying cells (red gate), suggesting CVA21 increases monocyte survival within PBMC.

CVA21 predominantly upregulates CD86 on monocytes and induces IFNs from PBMC

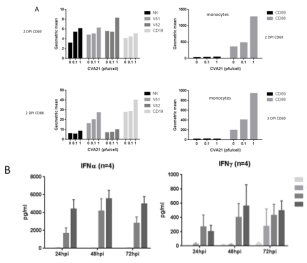

Figure 2: A) Representative PBMC donor showing phenotypic activation by CVA21 is restricted to upregulation of CD86 on monocytes (in contrast to other RNA OV measles, reovirus); B) Interferon secretion by CVA21-activated PBMC (no significant secretion of IL10, IL28/29, TNFa – not shown).

CLL cells, but not AML, express CVA21 receptors and are killed by the virus

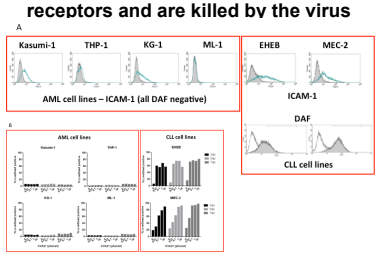

Figure 3: A) expression of ICAM-1 and DAF on AML and CLL cell lines; B) % death at 3, 5 and 7 DPI of AML and CLL cell lines after treatment with CVA21.

CVA21 cytotoxicity against CLL is reduced in the presence of human serum

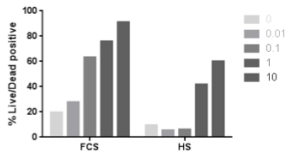

Figure 4: % death of MEC-2 CLL cells 3 days after treatment with CVA21 in fetal calf serum (FCS) versus human serum (HS).

Results

- Figure 1 demonstrates that PBMC variably express the CVA21 receptors ICAM-1 and DAF, but are not killed by the virus. The monocyte gate within PBMC expands after treatment with CVA21, with fewer dead/dying cells, suggesting that the virus increases monocyte survival within PBMC.
- Figure 2 shows that phenotypic activation of cells within PBMC by CVA21, as measured by CD69 and CD86, is mainly restricted to upregulation of CD86 on monocytes. This is associated with secretion of IFNα and IFNγ by CVA21-infected PBMC.
- Figure 3 illustrates that CLL cells, but not AML cells, express both ICAM-1 and DAF. This correlates with their sensitivity to killing by the virus.
- Figure 4 demonstrates that killing of CLL cells by CVA21 is reduced in the presence of human serum

Conclusions

1. **CVA21 infects but does not kill PBMC, with expansion of monocytes, which acquire a more antigen presenting cell phenotype, in the context of IFNα and IFNγ secretion.**
2. **CLL cells, but not AML, express both ICAM-1 and DAF, and are killed by CVA21, although cytotoxicity is reduced in the presence of human serum.**
3. **CVA21 is a potential treatment for CLL, but the consequences of systemic administration may differ from other RNA oncolytic viruses, which trigger a stronger early innate immune response.**

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