Master's Thesis Engineering Technology 2018-2019

Modulation of adenovirus replication by antiviral and immunosuppressive agents

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1. Introduction

Human **adenoviruses (AdVs)** are widespread pathogens transmitted by aerogenic or direct contact. In healthy persons, AdVs infections mostly cause self-limiting respiratory disease, diarrhea and ocular infections. At the same time, AdVs can be **life-threatening** for immunocompromised individuals. Despite the clear medical need, there is **no approved anti-AdV therapy.**

2. Aim of study

Investigate whether and how antiviral, immunosuppressive or cytostatic drugs mediate the AdV life cycle. A series of these drugs were tested in AdV-infected human embryonic lung (HEL) cells and A549 cancer cells, to assess their **antiviral (or proviral) effect on AdV replication**.

3. Materials and Methods

- Different chemical compounds were tested on AdV-infected HEL cells and A549 cancer cells [Figure 1].
- Virus replication was monitored by:
  - Immunofluorescence (IF)
  - cell viability assay
  - qPCR for viral DNA.
- Determination of the expression of two well-known AdV receptors (CAR and CD46) was evaluated by Fluorescence-Activated Cell Sorting (FACS). All data analysis was obtained using the FlowJo software.

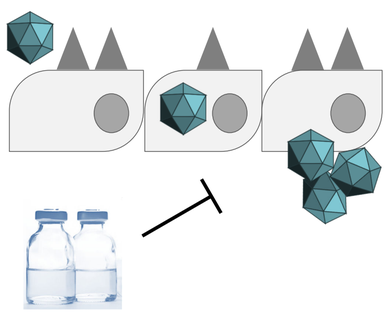

Figure 1. Antiviral, immunnosuppressive and cystostatic agents tested against AdV infected cells

4. FACS results and discussion

✓ A **significant signal** for **CD46** was obtained [Figure 2].

Expression of receptor in both cell lines.

✓ **No detection for CAR**
as the peak did not showed a clear shift compared to istotype control staining.

<u>Impact value:</u>
Tool to investigate entry inhibitors interfering with the AdV-CD46 interaction.

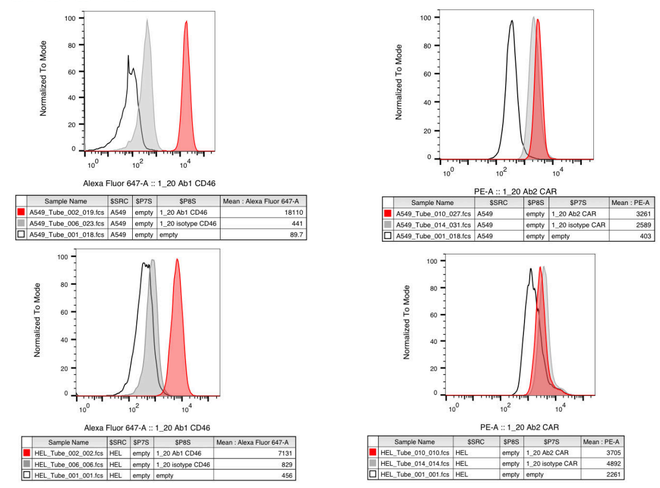

Figure 2. FACS histograms of A549 (first row) and HEL (second row) cells for detection of CD46 (left panels) and CAR (right panels). An antibody dilution of 1/20 was used. The peaks represent: in red, anti-CD46 (left) or anti-CAR (right) staining, in grey: isotype control staining, and in black: unstained control.

5. Overview of antiviral activity

Table 1. Anti-AdV activity and cytotoxicity of most promising compounds evaluated in HEL cell cultures

Compound	Working mechanism	[unit]	Antiviral EC_{50}[A] AdV-2 MTS	AdV-2 visual CPE score	AdV-3 MTS	AdV-3 visual CPE score	AdV-7 MTS	AdV-7 visual CPE score	AdV-8 MTS	AdV-8 visual CPE score	AdV-9 MTS	AdV-9 visual CPE score	Cytotoxicity CC_{50}[B]	Cytotoxicity Minimum cytotoxic concentration[C]
Cytostatic agents														
Gemcitabine	Pyrimidine antagonist; Chain terminator when incorporated into DNA	µM	>100	>100	69	>100	67	41	>100	>100	2.6	5.3	10	1.3
Cytarabine	(idem)	µM	>100	86	>100	>100	78	>100	>100	73	>100	57	75	70
PMEG	(idem)	µg/mL	5.3	0.18	5.0	0.20	>10	0.22	>10	>10	>10	>10	>10	>10
5-Fluorouracil	(idem)	µM	85	34	54	56	73	20	>100	84	2.7	7.6	>100	>100
Cladribine	Purine antagonist; Chain terminator when incorporated into DNA	µM	3.5	1.9	0.12	0.41	/	1.8	>100	79	0.49	0.32	25	≥20
Clofarabine	(idem)	µM	14	0.47	5.5	0.41	0.031	0.70	0.63	0.080	0.040	0.36	5.3	2.7
Methotrexate	Inhibits the synthesis of DNA, RNA, thymidylates, and proteins	µM	>100	>100	0.10	0.060	4.8	0.22	0.56	0.45	0.060	0.080	>100	>100
MPA	Inhibits lymphocyte proliferation	µM	10	6.5	5.4	11	5.3	20	1.5	12	0.15	1.4	>100	>100
Immunno-suppressive agent														
Sirolimus	Blocking signal transduction of inflammatory cytokines and IL by inhibiting mTOR pathway	µM	7.1	4.5	≤0.0013	≤0.0013	≤0.0013	≤0.0013	0,02	≤0.0013	≤0.0013	≤0.0013	53	47
Leflunomide	Inhibitor of protein kinase activity and pyrimidine synthesis shows potential for CMV therapy	µM	>100	>100	>100	80	>100	82	66	70	≤0.0013	15	75	>100
Cyclosporine	Blocking signal transduction of inflammatory cytokines and IL by inhibiting calcineurin	µM	>10	>10	>10	>10	>10	>10	>10	1.4	>10	>10	51	≥20
Inhibitors of virus entry														
Dextran sulfate	Polyanionic compound with broad antiviral activity	µg/mL	36	16	0.71	1.9	1.5	1.3	83	>100	≤0.0013	4.0	>100	>100
$NMSO_3$	Sulfated sialyl lipid compound with broad antiviral activity	µg/mL	4.0	8.4	0.060	8.9	40	23	>100	82	0.010	38	>100	>100
Inhibitors of viral AdV polymerase														
Cidofovir	Broad DNA-virus inhibitor; approved for CMV therapy	µM	1.4	3.4	0.63	4.3	4.9	5.5	0.010	2.0	1.3	11	>100	>100
Zalcitabine	Inhibits HIV RT; approved for HIV therapy	µM	3.4	5.8	0.090	8.5	0.91	9.8	18	3.1	1.2	4.9	>100	>100
Alovudine	Inhibits HIV RT; development halted during clinical trials	µM	3.1	1.9	0.020	2.8	<0.0032	3.7	<0.0032	6.6	1.1	1.2	>100	>100

The shading indicates strong (dark orange) or moderate (light orange) antiviral activity.
[A] 50% effective concentration (EC_{50}), or concentration producing 50% inhibition of virus-induced cytopathic effect (CPE), as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay.
[B] 50% cytotoxic concentration (CC^{50}), as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[C] minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.

6. Immunofluoresence

HEL and A549 cells
infected with a green fluorescent protein (GFP)-encoding AdV-5 virus [Figure 3].

replication-incompetent
due to deletion of E1 and E3 genomic regions

prominent GFP signal
virus entry without cytopathic effect on cells

<u>Impact value:</u>
Tool to investigate antiviral compounds whether they act before or after late gene expression, including virus entry inhibitors.

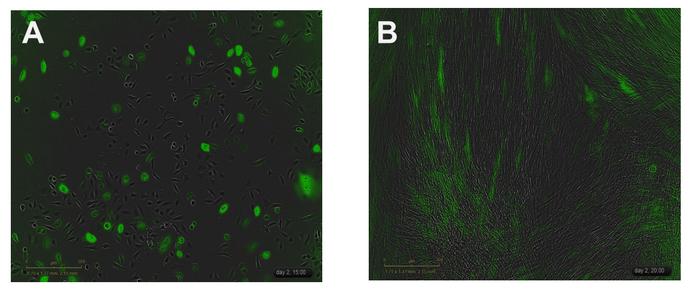

Figure 3. GFP signal in A549 (A) and HEL (B) cells infected with the AdV5-GFP (AVP011) vector 2 days post infection.

7. Conclusion

Our findings provide a **basis to further investigate the anti-AdV activity** of a number of immunosuppressive/cytostatic molecules, the most interesting one being **sirolimus**. To understand the different impact of sirolimus on AdV replication in the two cell lines, **more research is needed**. In the near future, the methods developed in this thesis, will be particularly useful to confirm the anti-AdV activity of the hit compounds, and reveal their basic anti-AdV mechanism of action.

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