Inflammatory profile of AAV6 in a pig and human model

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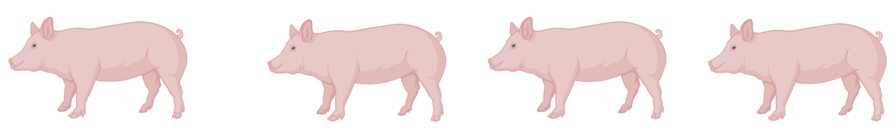
Introduction / Background

Adverse effects related to inflammatory reactions compromise the safety and long-term efficacy of retinal gene therapy. AAV6 has previously shown promise for transduction of immune cells. We examined retinal immune cell activation following subretinal injection of AAV6, comparing it to the pan-retinal vector, AAV8 in Yucatan minipigs. We further compared the effects of these vectors on a culture of microglial cells derived from human iPSCs. An overt inflammatory response was found in 2 of 3 pigs injected with a moderate to high dose of AAV6-CAG.EGFP while pigs injected with AAV8-CAG.EGFP or PBS did not show clinical effects. A proliferation of invading phagocytic mononuclear cells were found in AAV-injected eyes. Cytokine secretion following AAV6 and AAV8 treatment of microglial cultures was subsequently evaluated.

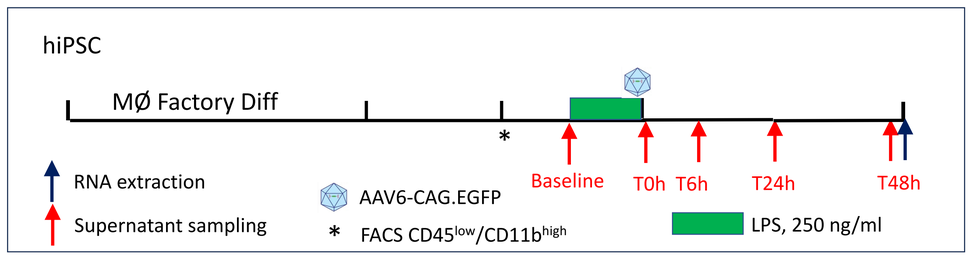
Materials & Methods

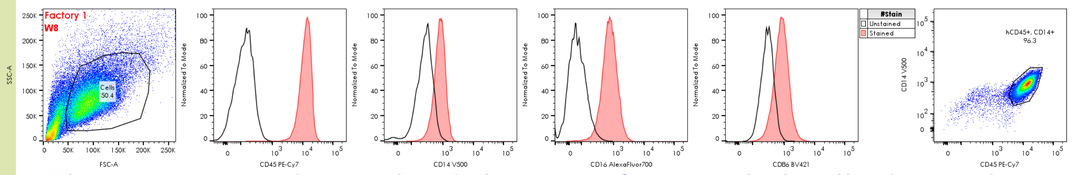
Subretinal bilateral injection of rAAV (4,6e11 total vg/eye) was performed in the eyes of minipigs (6-month- old, 20kg):



AAV6-CAG.GFP n = 3 | **AAV8-CAG.GFP n = 1** | **BSS n = 2** | **Non-injected n = 1**

A subconjunctival injection of 0.5ml of corticosteroid (methylprednisolone, 40mg/ml) was performed at the end of the procedure. Optical coherence tomography (OCT) and IHC on flat- mount retinas was used to assess outcome.





The *in vitro* study involved the use of microglial cells derived from human iPSCs tested with AAV at MOI of 1E5. RNA from cells were analysed by RT-qPCR and cytokine secretion into culture medium was tested by an electrochemiluminescence-based multiplex ELISA, MSD (Mesoscale Discover assay).

Conclusion

The results highlight the risk for retinal inflammation and the need to further study the serotype-specific response of microglial cells in rAAV-based gene therapy studies. The intracellular response of microglia to AAV transduction may result in a specific cytokine signalling cascade. While we find AAV6 expressing EGFP to be toxic in the pig retina, a pronounced cytokine secretion was not found in vitro.

Results

Retinal structure was assessed by OCT of eyes before injection and 1 month after injection. Iba1+ cells were counted on confocal slices of flat-mount retinas from the pig eyes in 6 random squares of 500µm side length in the injected zone (IZ), peripheral non- injected zone (NIZ) and macular NIZ.

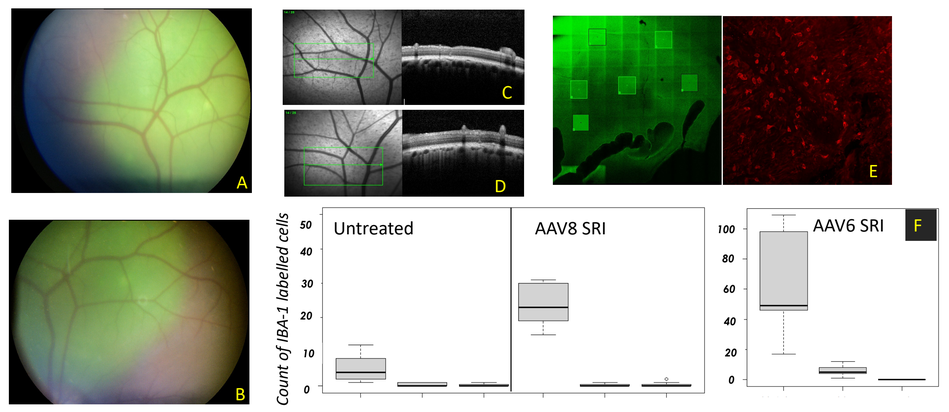


Fig 1 Effects of AAV6 compared to AAV8 in a pig retina: Colour fundus photographs of retinas injected with AAV6-CAG.EGFP (**A**), AAV8-CAG.EGFP (**B**) show transgene spread while the structure for AAV6 (**C**) and AAV8 (**D**) are shown with a proliferation of fluorescent phagocytic cells (**E**). IBA1+ cells counted on retinal flat-mounts were more numerous in IZ of AAV6-injected pig (61.3 +/- 35.0) than AAV8-injected pig (mean 23.5 cells/square +/-sd 6.3) (**G**). *SRI: subretinal injection*

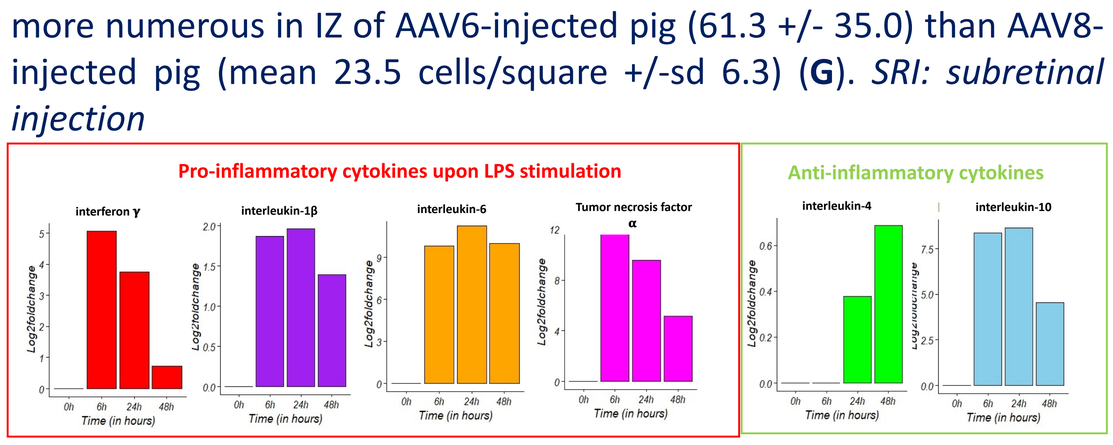


Fig 2 Validation of inducible human microglial (iMGL) culture model: cytokine secretion upon LPS induction show the expected profile.

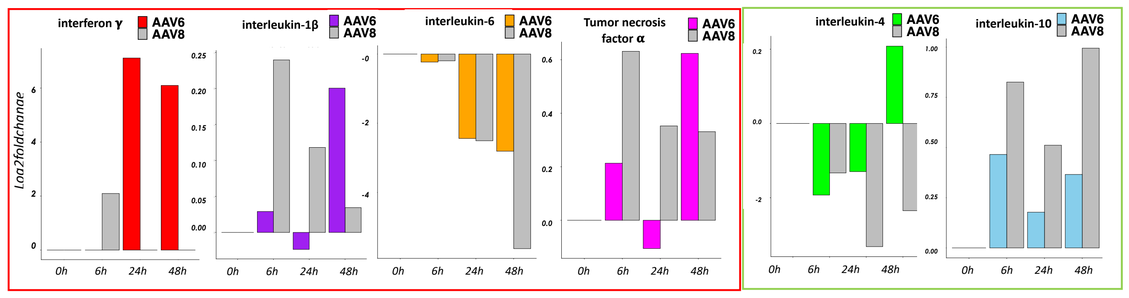


Fig 3 Effect of AAV–CAG.EGFP treatment on iMGL culture model: cytokine secretion upon AAV treatment.

	APO E	CCL2	IL6	CD47	CD68	MFGegf8	TMEM119	VEGFa
LPS	0,14	7,41	-0,06	3,28	0,18	-1,21	1,04	1,84
Adenovirus	0,24	1,02	0,73	0,87	0,28	0,27	0,24	0,92
AAV6	-0,09	0,56	-0,29	0,25	0,20	-0,25	0,55	-0,67

Log RQ (GOI/TBP)

Note that…

No significant GFP expression in iMGL cells was observed for any AAV serotype tested!

Fig 4 Effect of AAV6–CAG.EGFP treatment on iMGL gene expression: cellular expression measured 24 hours after transfection and shown normalised to untransfected cells.

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