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22nd January 2010

Dr Christine Hemmings
Honorary Secretary
Board of Education
The Royal College of Pathologists of Australasia
Durham Hall
207 Albion St
Surry Hills, NSW 2010
AUSTRALIA

Dear Dr Hemmings,

Please find attached my final report for the RCPA Research Award. Please accept my apologies for the delay in submitting this report but I was awaiting the results of some final experiments. As per the conditions of the award I will be preparing a manuscript to submit to *Pathology* for consideration for publication. If you require any further information please feel free to contact me.

Yours sincerely,

Dr James Ussher

Final Report for RCPA Research Award

Project Title: **New recombinant adeno-associated virus vectors as potent T cell vaccines**
Investigator: **Dr James Ussher**
Supervisor: **Dr John Taylor**
Co-Supervisor: **Assoc Prof Rod Dunbar**

Aims of the project:

1) To optimise recombinant adeno-associated virus as a vaccine vector by developing a vector that stimulates a strong innate immune response and thereby the maturation of transduced antigen presenting cells leading to improved T cell stimulation.
2) To generate rAAV vectors that restrict expression of antigens and "danger signals" to dendritic cells through the use of a DC-specific promoter.

Results:

DNA sequences encoding either a 40bp hairpin RNA (DS40), TRIF, an adaptor molecule in the MYD88-independent Toll-like receptor signalling pathway, or VISA (also known as IPS-1, CARDIF, and MAVS), an adaptor molecule in the RIG-I/MDA-5 signalling pathway, were cloned into an expression cassette driven by the immediate/early CMV promoter in pcDNA3.1 (pcDNA3.1/DS40, pcDNA3.1/TRIF, and pcDNA3.1/VISA respectively). The ability of pcDNA3.1/DS40, pcDNA3.1/TRIF, and pcDNA3.1/VISA to stimulate NFKB and interferon regulatory factor 3 (IRF3) signalling pathways was assessed by co-transfection with reporter plasmids into human embryonic kidney (HEK) 293 cells. pcDNA3.1/TRIF and pcDNA3.1/VISA were found to stimulate both signalling pathways, while no upregulation of either pathway was seen with pcDNA3.1/DS40. Although pcDNA3.1/TRIF led to stronger reporter expression than pcDNA3.1/VISA, given the packaging restraints of rAAV it was decided to proceed with VISA as it is 840bp smaller than TRIF.

Plasmids encoding a bicistronic vector were created by cloning the expression cassette from pcDNA3.1/VISA into the AAV vector backbone plasmid. Various configurations of bicistronic vector were generated. Dual immediate/early CMV promoter plasmids with either eGFP or VISA at the 5' position were cloned. In addition plasmids were cloned with either eGFP or VISA driven by the immediate early CMV promoter and the other gene driven by the internal ribosomal entry site (IRES) of the encephalomyocarditis virus. Plasmids were transfected into HEK 293 cells and eGFP expression detected by flow cytometery and activation of the NFKB and interferon regulatory factor 3 (IRF3) signalling pathways assessed as before. Similar expression of eGFP and activation of NFKB and IRF3 was seen following transfection with either a dual promoter bicistronic plasmid or a monocistronic plasmid. A modest reduction in activity was seen when eGFP or VISA was driven by the IRES, but activity was still greater than 40% of that seen following transfection with the monocistronic plasmid.

Recombinant AAV encoding either the dual promoter or IRES-containing bicistronic vectors were produced. Infection of HEK 293 cells confirmed that the virus was infectious as determined by eGFP expression. Monocyte-derived dendritic cells (MoDCs) from 4 donors were infected at a multiplicity of infection (MOI) of $1x10^4$ with either the bicistronic or monocistronic rAAV vectors. In addition a vector expressing eGFP under the transcriptional control of a CAG/WPRE promoter/enhancer element was included. Unfortunately eGFP expression was not detected when driven by the immediate/early CMV promoter in either a bicistronic or monocistronic vector. In contrast strong eGFP expression was detected in MoDCs when eGFP expression was driven by CAG/WPRE, with 1.75 to 12.5% of cells expressing eGFP. Infection of HEK 293 cells confirmed that the all vectors were infectious, although a transduction assay in HeLa cells found lower rates of eGFP expression with a CMV promoter compared with CAG/WPRE.

The activity of the antigen-presenting cell-specific promoter, HLADRa, was also assessed. The HLADRa promoter was cloned into the AAV vector backbone and its function and specificity confirmed by transient transfection of a HLADRa-positive lymphoblastoid cell line, LG2, and the HLADRa-negative cells lines HeLa and HEK 293. Vectors were made with either eGFP or luciferase expression under the transcriptional control of HLADRa. However, when MoDCs were infected with rAAV at a variety of MOIs eGFP expression could not be detected. Infection of MoDCs with luciferase-expressing vectors showed 20 fold less expression with the HLADRa promoter than with the CAG/WPRE promoter.

Therefore, problems of promoter strength need to be overcome. A bicistronic vector expressing eGFP and VISA under the transcriptional control of CAG/WPRE and an IRES is being developed. However, the utility of such a vector is limited by the packaging capacity of the vector. In addition other DC-specific promoters have been cloned and are being assessed for potency in MoDCs.

Dr James Ussher

Dr John Taylor

Assoc. Prof. Rod Dunbar