

Cytolytic DNA vaccines enhance immunity to hepatitis C virus.

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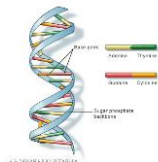
DNA vaccines

Advantages

- ❖ Simple and inexpensive
- ❖ Stable
- ❖ Can be deployed rapidly
- ❖ Individuals have no pre-existing immunity
- ❖ Can be used in multidose homologous regimen or heterologous prime/boost regimen
- ❖ Generate cell mediated and humoral immunity

Disadvantages.

- ❖ Suboptimal delivery
- ❖ Poor antigen expression
- ❖ Lack of localised inflammatory response
- ❖ Not effective in large animals and humans



Hypothesis

DNA vaccines which encode a suitable viral immunogen and a cytolytic protein will induce necrosis in vaccine targeted cells resulting in release of a range of DAMPs and cross presentation of the immunogen, and an increase in virus-specific immune responses.

Aim

To attract circulating DC to the site of vaccination and induce a degree of inflammation.

Outcome

Mimic live attenuated viral vaccines.

Factors associated with recovery from HCV infection

Rapid antibody response to HVR1 in E2

High titres of anti-E2 which inhibit E2 binding to CD81 - more recently, neutralising antibody to HCVpp

Vigorous CD4+ & CD8+ cell response to NS proteins esp NS3 & NS5. Th1 cytokine production.

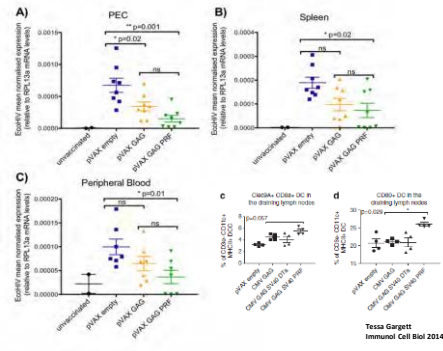
Strong proliferation of HCV-specific T cells (Nascimbeni 2003; Shata 2002)
Higher frequency of intrahepatic CD8+ T cells expressing perforin (Wabanabe 2010)

Early, sustained IFN-γ production by CD4+ and CD8+ T cells (Thimme 2002; Spada 2004)

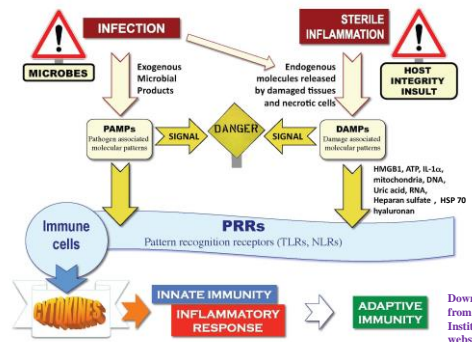
Innate immune responses, especially NK cells (Alter 2011)

HLA-DR+, CD38-, CD8+ T cells (Zubkova 2014)

A DNA vaccine which encodes Gag and a cytolytic protein provides greater protection against challenge with EcoHIV.



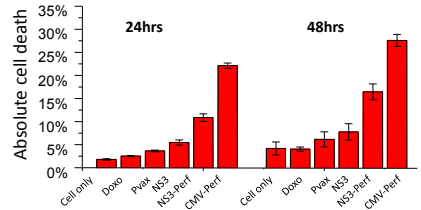
PAMPs and DAMPs trigger innate immune responses leading to adaptive immunity



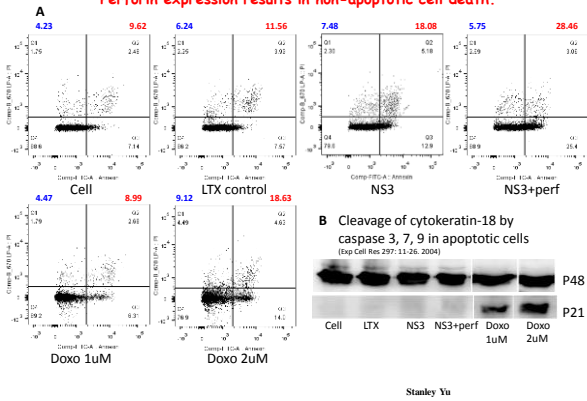
DNA vaccines used to examine the effect of perforin expression

pVAX	CMV prom		
pVAX-NS3	CMV prom	NS3	
pVAX-NS3-PRF	CMV prom	NS3	SV40 prom Perforin
CMV-PRF	CMV prom	Perforin	

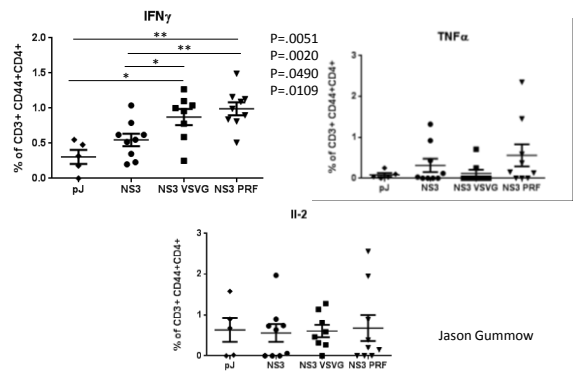
Perforin expression results in HEK 293T cell death in a LDH release assay



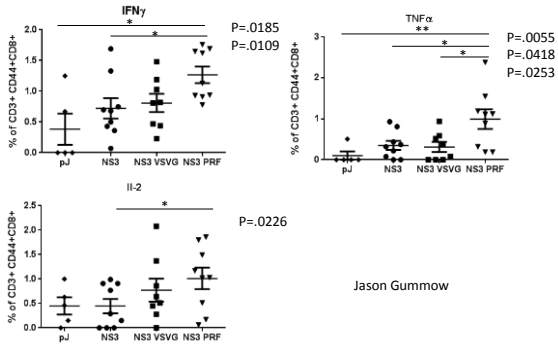
Perforin expression results in non-apoptotic cell death.



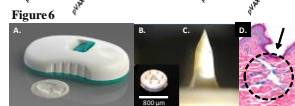
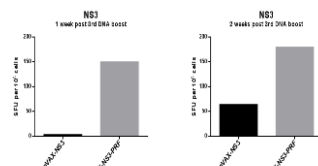
ELISpot analysis of CD3+ CD4+ CD4+ cells in vaccinated mice



ELISpot analysis of CD3+ CD4+ CD8+ cells in vaccinated mice



Cytolytic gene technology increases cellular immunity in DNA vaccinated pigs



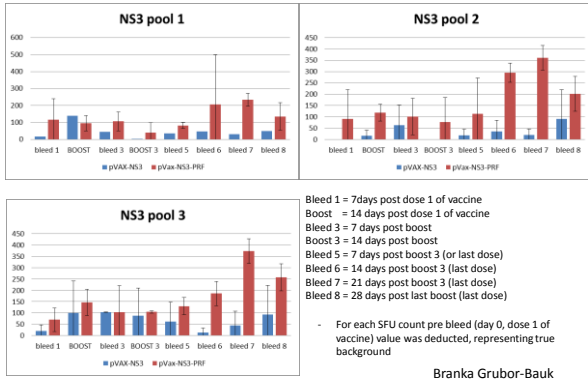
White landrace pigs were vaccinated with 300ug DNA encoding the HCV NS3 protein +/- PRF on 3 occasions, 2 weeks apart. 7-14 days later, PBMC were stimulated with NS3-specific peptides and IFN-gamma measured by ELISpot.

The DNA was delivered via the ID route by a microneedle device to ensure reproducible delivery

Branko Grubor-Bauk
Stanley Yu
In preparation

FLUGEN INC, Wisconsin, USA

PIG PBMC data: IFN-g Elispot: PIGS vaccinated with 300ug DNA



Branka Grubor-Bauk

Conclusions

The efficacy of DNA vaccines can be improved the induction of necrosis in vaccine-targeted cells- this results in more rapid and higher responses

The technology is equally applicable to replication defective viral vectors eg. adenoviruses.

Not all cytolytic proteins are equal.
 Perforin induces non-apoptotic cell death-most likely necrosis
 There is a balance between the level and timing of expression of the immunogen and the cytolytic protein

PCT/AU2013/000509. Held by University of Adelaide.

Clinical trial*, 2015 HCV genotype 3-infected individuals.

	Group 1	Group 2	Group 3	Group 4
Anti-PD-1 antibody &/or anti-CTLA-4	+	-	+	-
Canonical DNA	-	+	-	-
DNA + Perforin	-	-	+	+

With Ian Roberts-Thomson, GE, TQEH, Adelaide

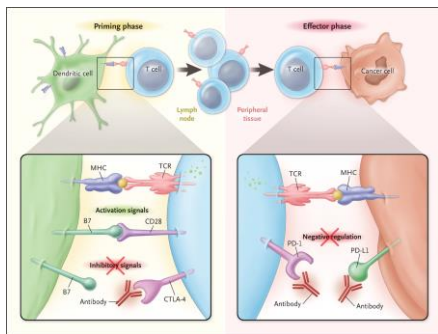
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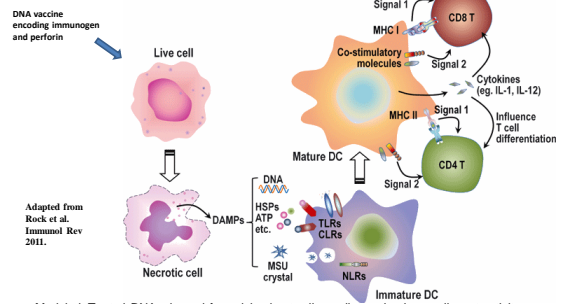


Blockade of PD-1 or CTLA-4 Signaling in Tumor Immunotherapy.



Ribas A. N Engl J Med 2012;366:2517-2519.

HCV/HIV immunogens released from necrotic cells are cross presented by dendritic cells which are matured by DAMPs



Marichal, T. et al. DNA released from dying host cells mediates aluminum adjuvant activity. *Nat Med* (2011)
 Zhang, J.G. et al. The Dendritic Cell Receptor Clec9A Binds Damaged Cells via Exposed Actin Filaments. *Immunity* (2012).