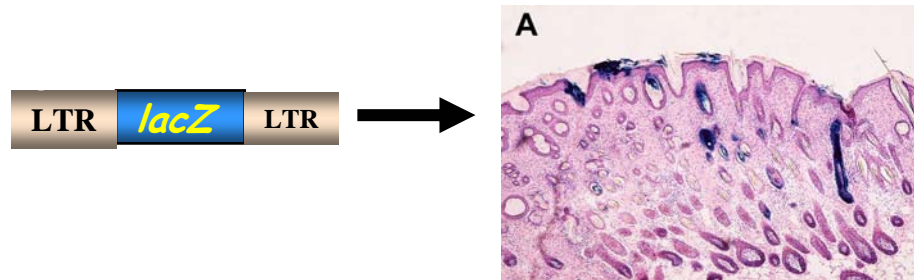


# Immunological Barriers in Molecular Therapy Targeted to Skin

Soosan Ghazizadeh, Ph.D.  
Department of Dermatology  
Columbia University Medical School

# In vivo RRV-directed gene transfer to skin

(Ghazizadeh, et al 1999, *Gene therapy* 6:1267-1275)



## Transgene expression

2 weeks

3 weeks

40 weeks

**Immune-competent**

+

+

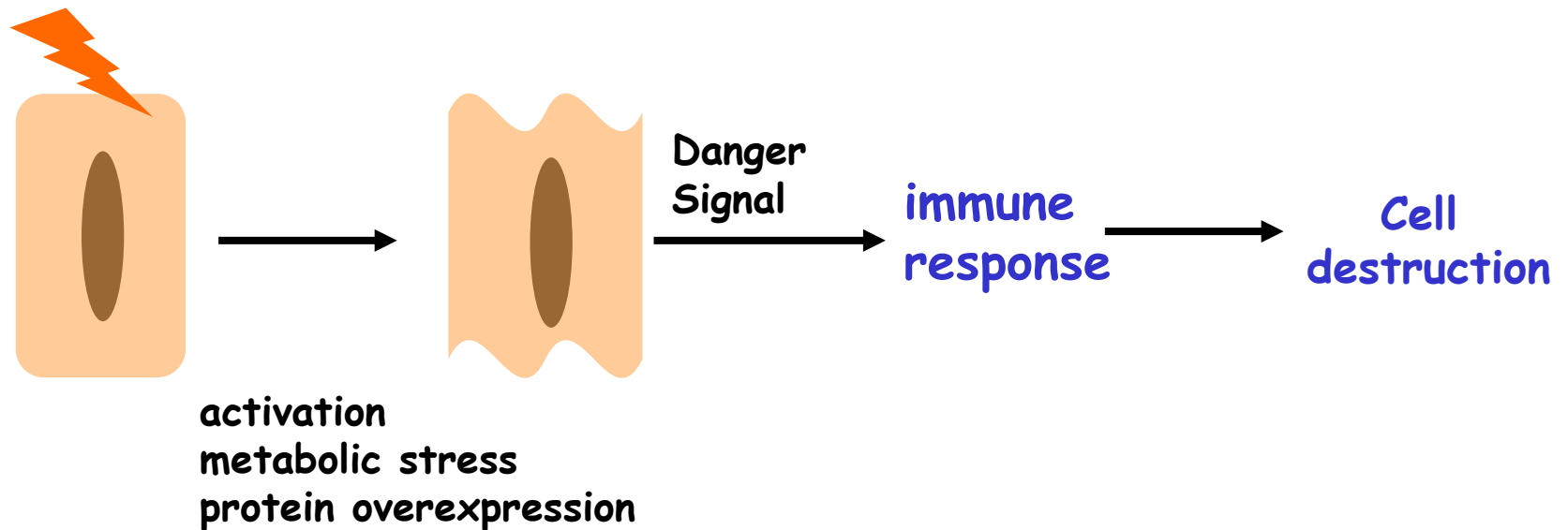
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Immune responses may limit the effectiveness of gene therapy

1-Transduce Stem cells

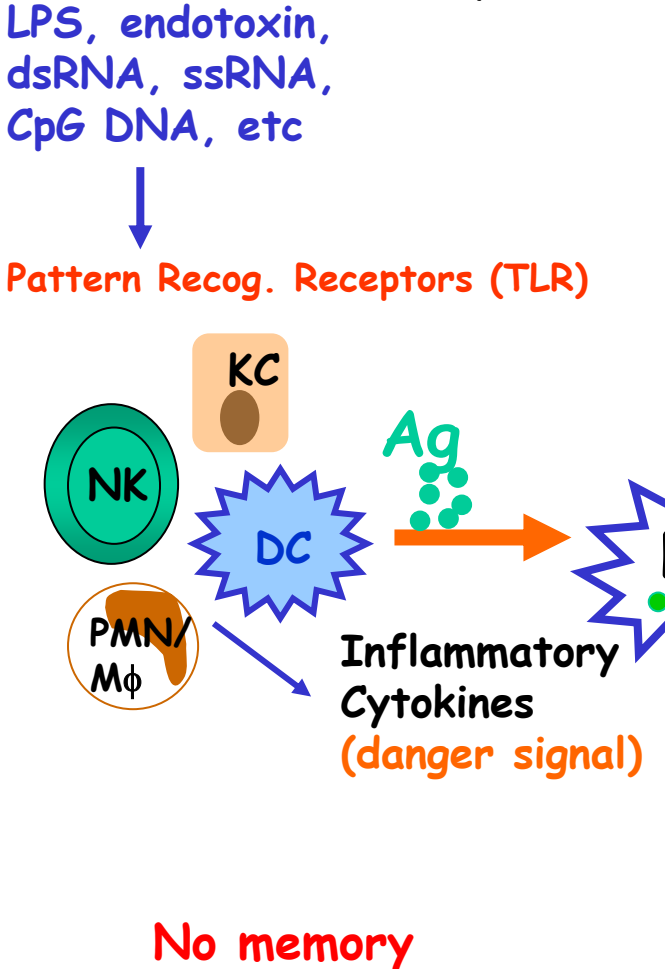
2-Persistent transgene expression

# Delivery of macromolecules often alters the state of target cell



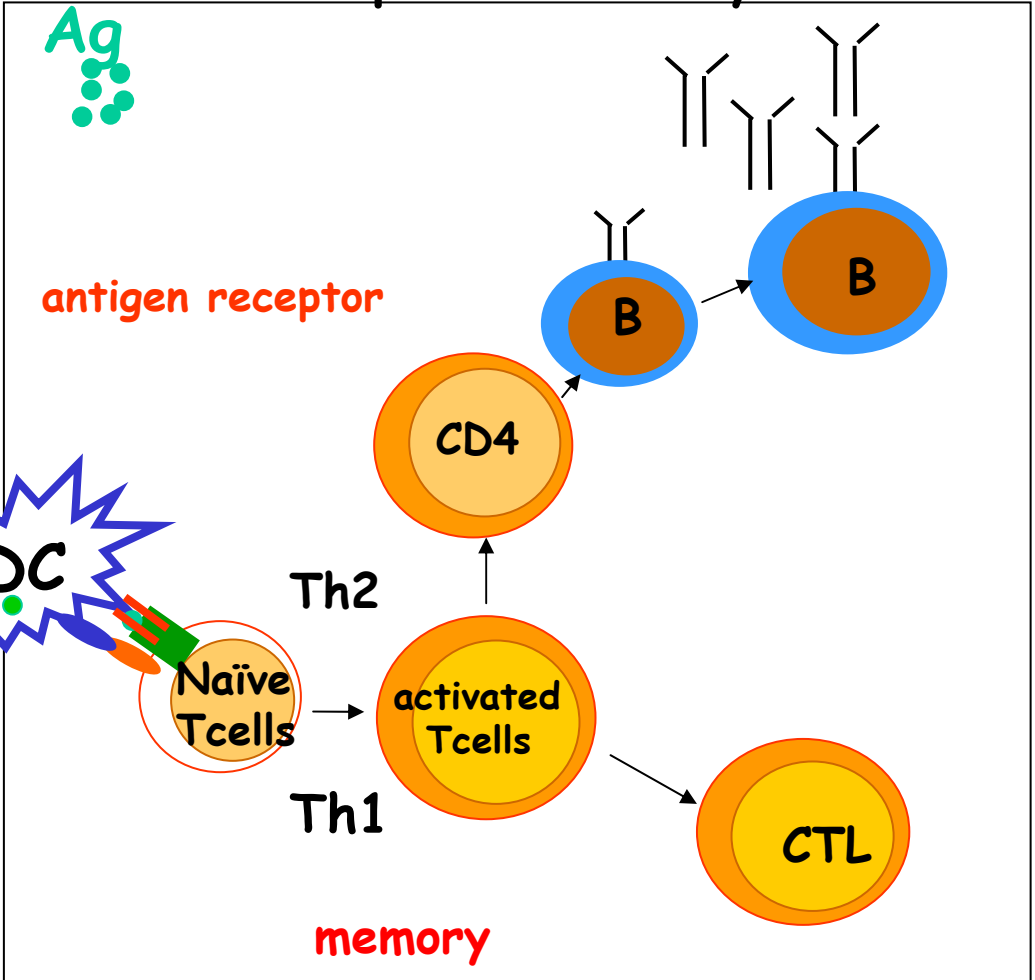
# Two Arms of Immune System:

## Innate Immunity



hours

## Adaptive Immunity



days



**Immune responses in molecular therapy are influenced by:**

- 1. Nature of the molecule being transferred**
- 2. Target tissue and tissue compartments**
- 3. Delivery methods**

## Immune responses must be considered in developing molecular therapy protocols:

- What type of immune responses are generated?
- How can we control/eliminate unwanted responses?

# What are the immune mediators of transgene loss following in vivo gene transfer?

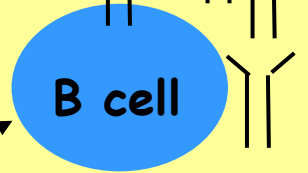
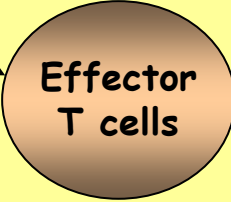
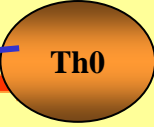
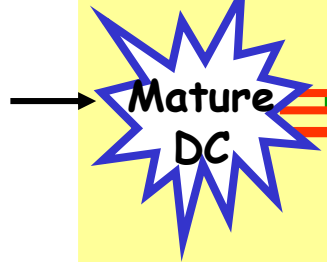
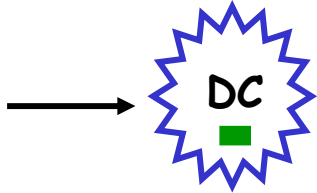
Ghazizadeh S, Kalish RS, and Taichman L. (2003) Molecular Therapy, 7: 296- 303.

Mouse strain	C57BL6	
Serum IgG	+	
CD8+ cells	+	
CD4+ cells	+	
Transgene expression	lost	

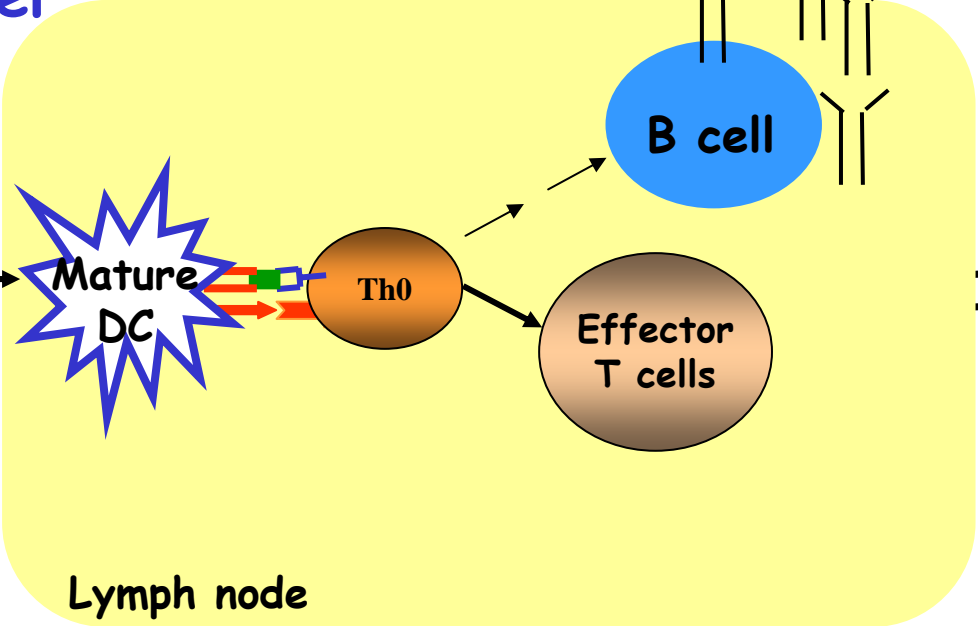
1. Loss of transgene is mediated by transgene-specific responses
2. Either CD4 or CD8 T cells could mediate clearance of transduce cells
3. Simply blocking one key player may not be sufficient

# In vivo gene transfer

Direct presentation

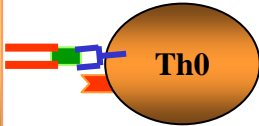
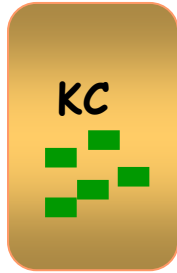


Immunity



Lymph node

Cross-presentation



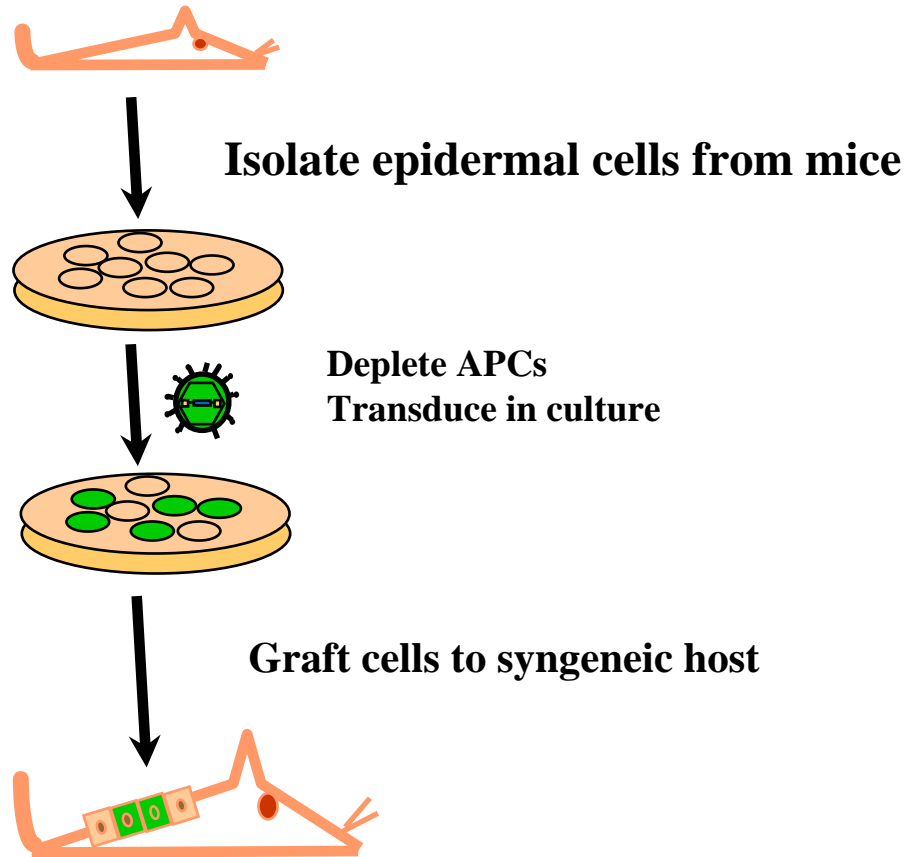
Ignorance

# Ex vivo gene transfer



# Host responses following *ex vivo* gene transfer to KC

(Lu and Ghazizadeh, 2005, *Exp. Dermatol.*, 14:727)

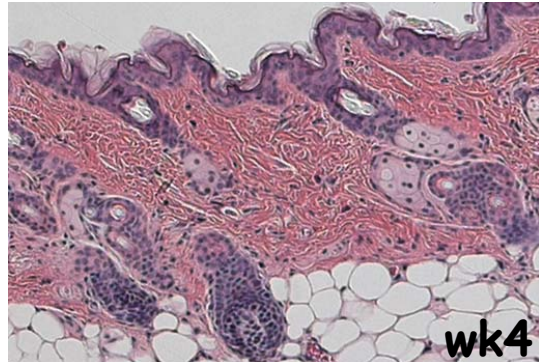
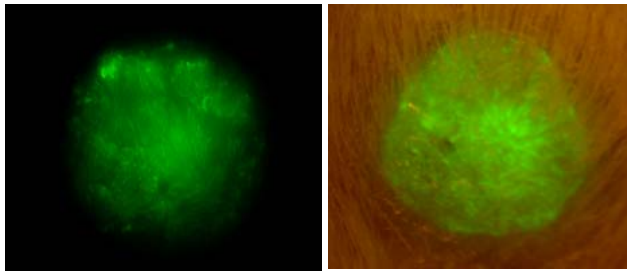


# Loss of transgenic cells by transgene-specific immunity

## GFP-tolerant

4 wk

20 wk

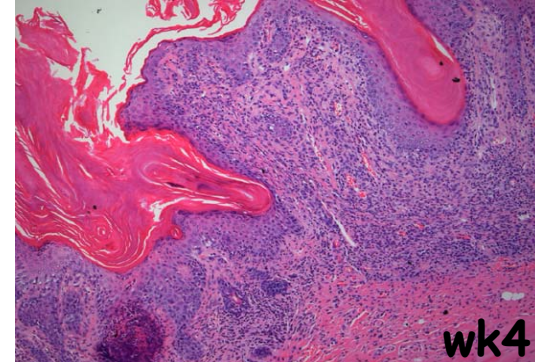
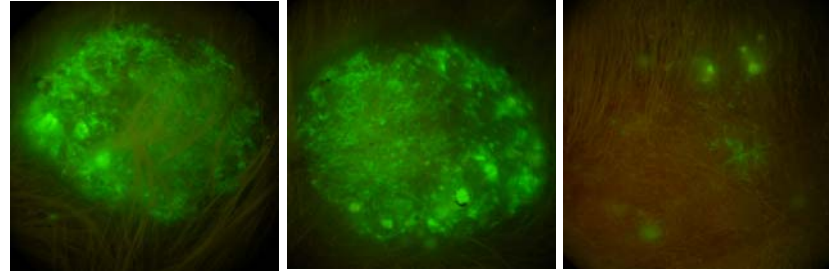


## Normal Mice

3 wk

4 wk

5wk



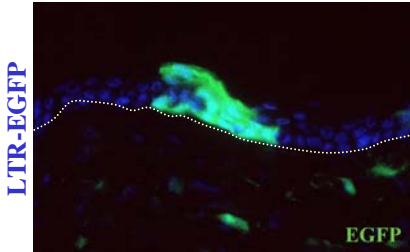
## Characterization of transgene-specific immune responses:

	<u>In vivo</u>	<u>Ex vivo</u>
Tissue infiltrate:	Lymphocyte	Eosinophils
Serum IgG isotype:	IgG2a	IgG1
Cytokine profile:	IFN- $\gamma$	IL-4
In vitro CTL activity:	Yes	NO
CTL memory:	Yes	NO
	<b>Th-1</b>	<b>Th-2</b>

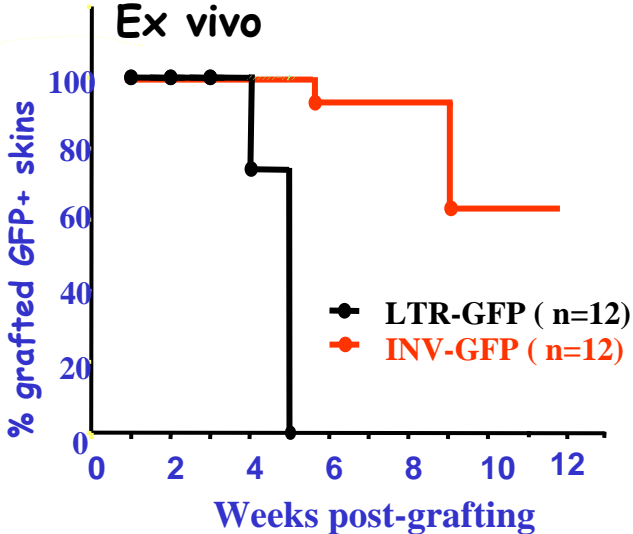
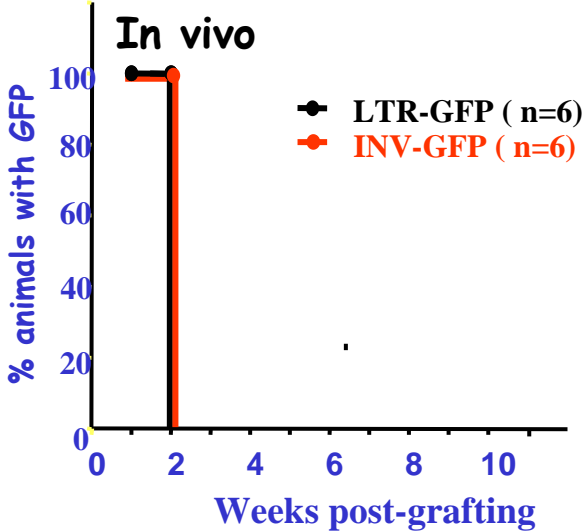
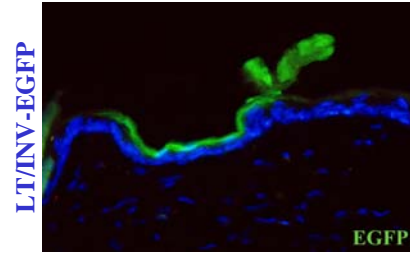
Immune responses are distinct between in vivo and ex vivo approaches to gene transfer

# Targeting transgene to suprabasal compartment of epidermis

**LTR-GFP**

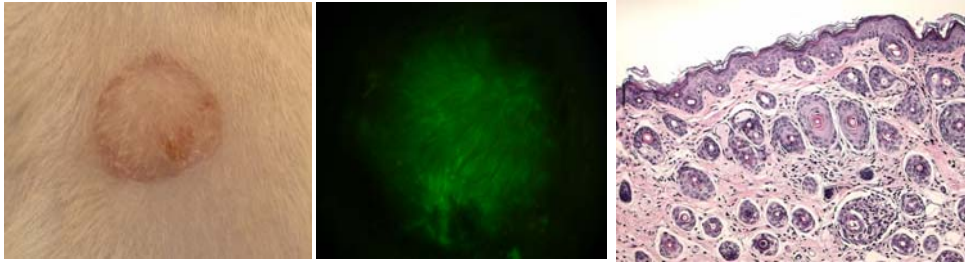


**INV-GFP**



# Tissue Responses

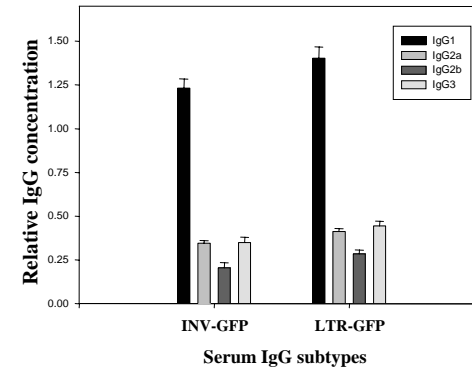
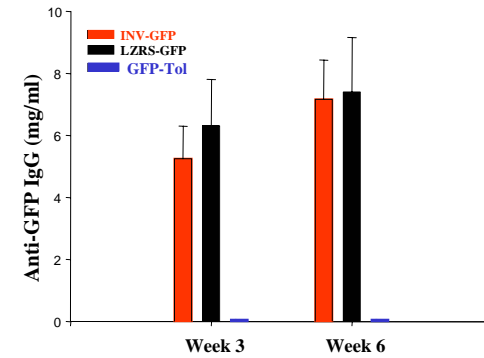
## INV-GFP



## LTR-GFP



# Humoral responses



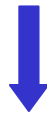
Suppression of antigen-specific tissue responses when transgene targeted to suprabasal KC

A potential strategy to control unwanted responses

## Conclusion:

- Immune responses are a **challenge**, not an impassible barrier

Need to develop strategies to suppress unwanted responses



type of immune response

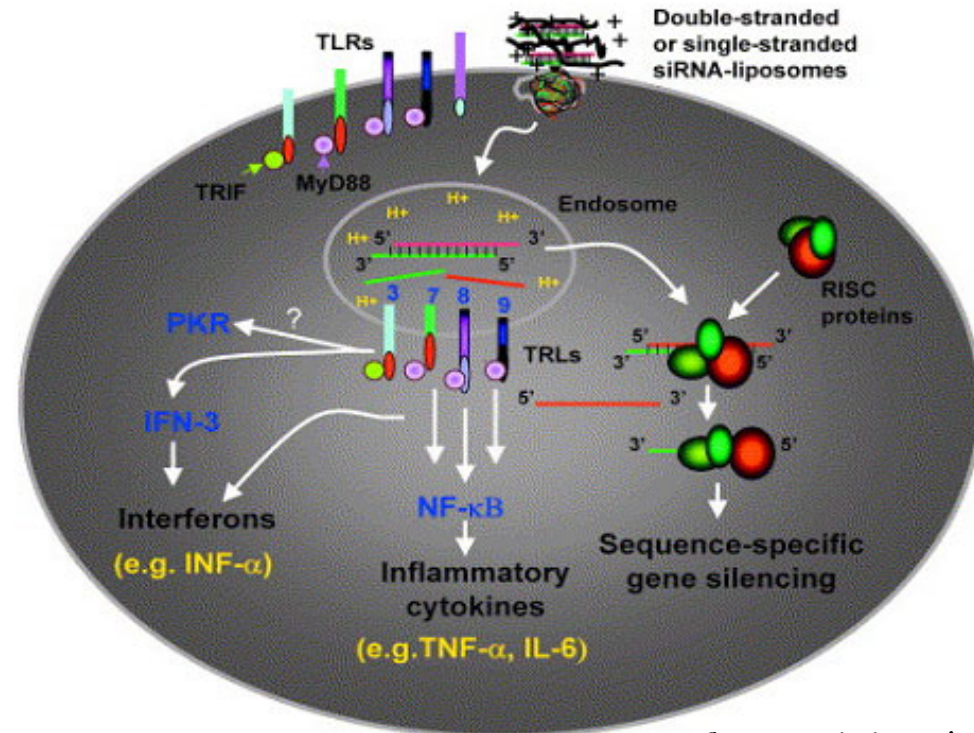


therapeutic approach



Disease

- Dominant disorders:** Expression of a mutant allele
- Selective suppression by siRNA, antisense, oligos,
- induction of innate immunity (TLR)
- low efficiency of therapy



Current opin immuol

## Focus:

### controlling innate immunity

- Alter molecules to make them less immunogenic
- Corticosteroids
- Anti inflammatory/cytokines
- New anti-innate immunity therapeutics

**Recessive disorders:** Loss or reduced gene expression

- introduction of normal cDNA/protein
- induction of innate immunity
- therapeutic protein = neoantigen
- induction of adaptive immunity/loss of transgenic cells

**Focus: Modulate adaptive immune responses**

- Control tissue inflammation/injury
- Dissociate innate and adaptive immunity at DC
- Immune suppression by blocking co-stimulation
- Induction of regulatory cells/tolerance



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